ISSN: 2347-467X, Vol. 13, No. (1) 2025, Pg. 258-270

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Assessing the Effects of Diverse Germination Methods on Red Rice Quality: A Physicochemical and Sensory Approach

ANDI NUR FAIDAH RAHMAN[1]*, MULYATI TAHIR[1], JALALUDDIN HADDADA[2], RASYIDAH BAKRI[3], AMIRUDDIN HAMBALI[4] and ASMAUL HUSNAH[1]

[1]Department of Food Science and Technology, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia.
[2]Department of Mechanical Engineering, Hasanuddin University, Gowa, Indonesia.
[3]Department of Agribusiness, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia.
[4]Department of Agricultural Technology Education, Faculty of Engineering, Makassar State University, Makassar, Indonesia.

Abstract

Germination process is a crucial determinant of rice quality, which may be reduced due to improper post-harvest treatment. Therefore, this study aimed to determine the effect of red rice (RR) and unhusked dry rice (UDR) germination methods on the physicochemical and organoleptic properties of germinated RR. The approach consisted of two components, namely germination (RR and UDR) and soaking (water (W) and chitosan (C)), while sensory test was used to analyze rice quality. Physical analysis included color intensity, scanning electron microscopy (SEM), milled yield, and rice quality according to the Indonesian National Standard (INS). The chemical analysis included proximate levels, phosphorus, magnesium, gamma-aminobutyric acid (GABA), and antioxidant activity. The results showed that the highest organoleptic color, aroma, and texture were 3.37/5.00 (UDRW), 3.28/5.00 (UDRW), and 3.33/5.00 (UDRC). The physical analysis showed the highest percentage of head rice (87.63%) and milled yield (87.21%) was found in RRW. SEM test results showed that there were cracks on the surface of rice in all treatments, other properties were color intensity (a* 5.37) (RRC). The highest chemical analysis showed moisture content 11.53% (UDRW), ash 1.39% (Ungerminated rice), phosphorus 238.71 mg/100 g (RRC), magnesium 112.28 mg/100 g (RRC), protein 11.38% (Ungerminated rice), GABA 231.96 mg/kg (UDRC), fat 4.07% (UDRW), carbohydrates

Article History
Received: 21 November 2024
Accepted: 07 February 2025

Keywords
Chemical;
Chitosan;
Germination;
Physical;
Quality;
Red rice;
Unhusked Dry Rice (UDR);
Water.

CONTACT Andi Nur Faidah Rahman andinurfaidahrahman@unhas.ac.id. Department of Food Science and Technology, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia.

Doi: https://dx.doi.org/10.12944/CRNFSJ.13.1.17

78.34% (UDRC), and antioxidant IC_{50} 636.04 ppm (RRC). The conclusion of this study was variations in germination methods significantly affected the physical and chemical characteristics of germinated RR. There was a significant increase in the levels of GABA, phosphorus, magnesium, milled yield, and rice quality. However, there was a significant decrease in fat, morphological structure, and antioxidant activity. The results showed no significant effect on sensory, carbohydrate, protein, ash, and moisture content.

Introduction

Rice is a staple food that can provide energy for the body because it has a high carbohydrate content. Rice with high nutritional content is needed by consumers to maintain health and fulfill daily nutrients for the body.[1] Rice is divided into several types, namely white, red, black, and glutinous. Red rice (*Oryza nivara*) has a higher nutritional content than white because it is obtained without going through the shucking process, leaving the aromatic skin that attaches to rice endosperm.[1] However, the consumption of RR is still quite low, presumably due to the low organoleptic value, less favorable taste, less soft texture, and prolonged cooking process compared to white rice.[2]

The quality of rice produced is potentially influenced by the post-harvest handling process. In general, the processing of unhusked dry rice (UDR) reduces the nutritional content.[3] This is because in milling process, the husk and RR layers are removed, thereby reducing the content of amino acids including lysine, fat, protein, and fiber as well as antioxidant activity beneficial to the body.[3] Post-harvest handling to increase the nutritional content of rice is mainly achieved through germination process. Germination is the initial process of forming a new individual in seed plants, where the embryo initially in a dormant condition, experiences physiological changes.[4] These changes are influenced by environmental conditions including water, light, air, and nutrient sources which then react with chemical compounds, leading to active growth of the embryo into a new plant.[5] Germination process starts with the imbibition phase which causes softening of the seed coat and an increase in enzymatic activity.[6] Water imbibition process stimulates gibberellin activity which then activates the α-amylase enzyme. This enzyme moves into the food reserves hydrolyzing starch into sugar which will be used as an energy source in germination process.[6] According to previous studies, germination potentially increases the content of nutrients, bioactive compounds, and organoleptics in rice. For example, bioactive compounds such as GABA (ɣ-aminobutyric acid) can increase 10 times compared to ungerminated grain or rice.[7]

The increase in GABA is caused by a stress response in plants due to lack of water or oxygen, which enhances the activity of glutamate decarboxylase (GAD) enzyme. GABA compounds have many health benefits including being able to lower blood pressure, improve sleep quality, control stress, inhibit cancer cell proliferation, cause diuretic effects, and help the recovery process of alcohol-related symptoms.[8]

Germination is strongly influenced by certain conditions, underscoring the need for elicitors in the form of chitosan to optimize the process. Chitosan is one of the biopolymers obtained from chitin derivatives through the deacetylation process using strong bases.[9] It is widely used due to the biodegradable, hygroscopic, non-immunogenic, antimicrobial, non-toxic properties as well as high biocompatibility.[10] The use of chitosan can improve germination process and increase several bioactive compounds.[11] In addition, the application as an elicitor potentially increases antioxidants, anthocyanins, and GABA levels in germinated rice. The use of chitosan speeds up germination process about 20 hours faster compared to without the use of chitosan which takes about 24 hours. Increasing germination process with chitosan can elevate water absorption in seeds. This is due to the large number of hydroxyl groups formed by the transfer of NH- and OH- electrons (from chitosan) as well as O groups (from starch) which facilitates the imbibition process. Germination with various methods is expected to increase the nutritional content of the rice produced so that it can be beneficial for health.[12] Therefore, this study aims to compare the increase in quality and physicochemical characteristics in the form of proximate, antioxidant, GABA, magnesium and phosphorus of red rice through various germination processes.

Materials and Methods

Materials

The materials used in this study include RR of an unknown variety obtained in the form of UDR with a moisture content below 14% and freshly harvested grain. This sample was obtained from Takalar Regency, South Sulawesi Province, Indonesia. UDR was then divided into two treatments, namely milling UDR into RR and UDR without milling process. Both types of materials were stored in a dry place to avoid damage during the storage process.

Methods

Germination of Unhusked Dry Rice (UDR)

Germination of UDR was carried out using the method described by Rahman *et al.*[13] The process started with soaking UDR in water at a ratio of 1:2 (w/v) for 48 hours followed by draining. The grain was allowed to rest in a gunny sack for 24 hours until the average height of UDR germination was around 0.1-0.2 cm. The sprouts were dried until moisture content of the grain reached 14%.[13,14] Finally, milling process was carried out on the grain to separate the husk and rice.

Germination of Red Rice (RR)

Red rice germination was carried out based on the method described by Rachma *et al.*[15] which started by soaking RR in water in a ratio of rice:water 1:2 (w/v) for 2 hours followed by draining. Rice was placed in a gunny sack for 20 hours until the average height of rice germination was about 0.1-0.2 cm. Subsequently, the sprouts were dried under the sun until moisture content of rice reached 14%.[13,14]

Germination with Elicitor

Germination using an elicitor in the form of chitosan referred to the method described by Maligan *et al.*[16] where a solution was prepared by dissolving chitosan with a concentration of 200 ppm into water that had previously been acidified using citric acid with 1% of water volume. UDR and RR were then soaked in the chitosan solution for 48 hours and 2 hours, respectively. Subsequently, rice was placed into gunny sacks, 20 and 18 hours for UDR and RR, respectively. The drying process was carried out until moisture content reached <14% and germinated UDR was milled to separate the husk.

Table 1: Completely randomized design (CRD) with 3×2 factorial

Treatment	Type of Germination Material	Soaking Type
A1	Not germinated UDR (Control)	-
A2	Not germinated Red rice (Control)	-
A3	Unhusked Dry Rice (UDR)	Water (W)
A4	Unhusked Dry Rice (UDR)	Chitosan (C)
A5	Red Rice (RR)	Water (W)
A6	Red Rice (RR)	Chitosan (C)

Experimental Design

This study used complete randomized design (CRD) with a 3×2 factorial arrangement (Table 1). The two factors consisted of the type of germination material and the type of soaking. The types of germination materials used were UDR and RR, while the type of soaker was water and chitosan with a concentration of 200 ppm (w/v).

Analysis

Sensory Testing

Sensory testing was conducted using 5 hedonic scales that have been explained in previous studies.[17] This testing was conducted by 25 panelists who are students of food science and technology at Hasanuddin University. The testing was conducted in a sensory room with a temperature of around 20-

22°C and humidity of around 40-60%. The variables tested include color, aroma, and texture based on the hedonic method. Texture was tested by breaking rice seeds, while the organoleptic test score was divided into five, namely:

1 = strongly dislike
2 = dislike
3 = neutral
4 = like
5 = very like

Analysis of Physical Properties of Sprouted Red rice

Measurement of Milled Yield

Measurement of milled yield was calculated based on the method described by Rahman *et al.*[13] This process was carried out by calculating the ratio of RR produced to the initial weight of RR milled.

Rice Quality Testing

Quality testing of germinated rice was carried out based on the criteria in Indonesian National Standard (INS) 6128-2020, including head (%), broken (%), and rice groats (%).[14] The observation of milled rice yield started with 100 g of rice separated manually to obtain head, RR broken, and groats. Subsequently, each separated rice was weighed and expressed in percent. The calculation was carried out using the formula:

$$\text{Head Rice} = \frac{\text{head rice weight}}{\text{initial rice weight}} \text{ x } 100\%$$

$$\text{Broken Rice} = \frac{\text{weight of broken rice}}{\text{initial rice weight}} \text{ x } 100\%$$

$$\text{Rice Groats} = \frac{\text{weight of rice groats}}{\text{initial rice weight}} \, x \; 100\%$$

Color Analysis

The color of germinated RR was observed following a method described by Purbasari, Dayani and Sari with slight modifications.[18,19] The assessment was carried out using a colorimeter by firing rice sample. The L* (RR lightness), a* (redness), and b* (yellowness) values were then obtained.

Scanning Electron Microscope

SEM testing was carried out based on the modified method by Setyaningsih *et al,*[20] and Mohammed and Abdullah.[21] determined seven points of the sample preparation area. A five-carbon tip block stage with a diameter of 25 mm and a height of 10.8 mm was prepared, then one whole grain was taken from points number 1 to 5 using tweezers and an iron spatula attached to the carbon block stage. The samples that had been attached were sprayed with air using an electric blower, and each was put into the preparation box according to the number of samples. The preparation box was placed into SEM (Scanning Electron Microscopy) testing room. Subsequently, the coating process or gold plating was carried out using the Smart Coater tool on each samples for ± 1 minute. In the next step,the stage block carbon tip that had been coated was inserted into the holder.

Chemical Property Analysis of Germinated Red Rice

Proximate Analysis

The proximate analysis included testing water, ash, protein, and fat content according to the Association of Official Analytical Chemists (AOAC) testing guidelines.[22] Meanwhile, carbohydrate levels were tested using the differential method.

GABA (Gamma-Aminobutyric Acid) Analysis

GABA levels in sprouted rice which started with making a standard solution into a test tube measuring 2 mL.[23,24] About 2 g sample was weighed into a 50 mL volumetric flask, dissolved with aquabides until the mark, followed by homogenization and filtration with a 0.2 µm GHP membrane filter. The sample was pipetted 500 µL into a 2 mL tube and added with internal standard AABA 2.5 mM and aquabides followed by homogenization using a vortex. Subsequently, 10 µL of standard solution or sample solution that had been added to the AABA internal standard was pipetted into the vial, added with Accq. Tag Flour Borrate Buffer 2A, and vortexed. The mixture was heated at 60°C and then cooled to room temperature. The last step was to inject the solution into UPLC system until the data was obtained and calculated. GABA levels in the sample were calculated using the ratio of the area between the analyte and the internal standard.

$$\text{Standard or sample ratio} = \frac{\text{Aspl}}{\text{AIS}}$$

$$\text{GABA} = \frac{\frac{\text{Sample ratio}}{\text{Standard ratio}} \text{ x } \frac{\text{C std}}{1000000} \text{ x MW x Vp x Df}}{\text{Wspl or Vspl}}$$

Description

Aspl = Area of GABA analyte
AIS ` = Internal area of AABA standard
MW = Molecular weight of amino acid
Cstd = Concentration of amino acid standard solution (pmol/μL)
Wspl = Test portion weighing weight (gram)
Vspl = Pipetting volume of test portion (mL)
Vp = Final volume of test solution (μL)
Df = Dilution factor

Phosphorus and Magnesium Analysis

Phosphorus/magnesium mineral testing was carried out based on the method outlined by the Association of Official Analytical Chemists (AOAC).[22] A standard series of metal mixtures of at least 6 concentrations were prepared, then a sample of 0.5-1 g was weighed and placed in a vessel. HNO_3 was added and the solution was allowed to stand for 15 minutes. The vessel was closed and deconstructed in a microwave digester. Furthermore, the results of the deconstruction were placed into a 50 mL volumetric flask and added with internal standard yttrium 100 mg/L, followed by dilution with aquabides to the mark and homogenized. The solution was filtered with a 0.20 μm RC/GHP syringe filter, and the intensity was measured with an ICP-OES system at a wavelength of 214.914 nm. Mineral content in the sample was calculated using a standard calibration curve with the line equation: Y = bx + a, and the formula:

$$\text{Phosphorus/Magnesium (ppm, mg L, mg Kg)} = \frac{\frac{\text{Aspl} - \text{a}}{\text{b}} \times \text{V} \times \text{Df}}{\text{Wspl or Vspl}}$$

Description

Aspl = Sample intensity
a = Intercept of the standard calibration curve
b = Slope of the standard calibration curve
Df = Dilution factor
V = Final volume of test solution (mL)
Wspl = Weighing weight of test portion (g)
Vspl = Volume of test portion pipetting (mL)

Antioxidant Content Analysis

Antioxidant testing was carried out based on the method described in a previous study.[25,26] In this method, 2.2-Diphenyl-1-picrylhydrazyl (DPPH) was divided into several concentrations of 100, 200, 300, 400, and 500 ppm, then measured at a wavelength of 517 nm to determine IC_{50}.

Statistical Analysis

The data obtained were processed using the general linear model test (Univariate) and One-Way Analysis of Variance (ANOVA) to determine the differences in each treatment. When differences were observed, further tests were carried out using the Duncan Multiple Range Test ($p \leq 0.05$) in the SPSS 25 application.

Table 2: Physical properties of red rice and germinated red rice

Treatment	Milled yield (%)	Head rice (%)	Broken rice (%)	Rice groats (%)	INS 6128-2020[14]
A1	71.43^{c}	82.63^{c}	8.02^{b}	9.33^{b}	Medium 1
A2	71.43^{c}	82.63^{c}	8.02^{b}	9.33^{b}	Medium 1
A3	70.07^{b}	69.7^{b}	13.97^{c}	16.33^{c}	Medium 2
A4	60.15^{a}	55.6^{a}	17.1^{d}	27.3^{d}	Medium 2
A5	87.21^{d}	87.63^{d}	7.33^{b}	5.03^{a}	Premium
A6	85.8^{e}	87.57^{d}	4.63^{a}	7.8^{b}	Premium

Results

Based on the results of testing the physical characteristics of red rice germination results which include milled yield, head rice, broken rice and rice groats can be seen in Table 2.

The results of the Analysis of variance (ANOVA) showed that the interaction between the type of germination material and the type of soaking had a significant effect on the milled yield and quality of germinated red rice (Sig. 0.000) at the 5% level, so Duncan's further test was carried out, and it was found that there were significant differences.

According to the results obtained, it can be seen that Milled yield of A1 and A2 treatments without germination was 71.43%, while UDR germination experienced a slight decrease 70.07% for A3 and 60.15% for A4. Red rice germination experienced an increase in 87.21 for A5 and 85.8% for A6 as shown in Table 2. Rice quality is divided into three categories based on INS 6128-2020, namely head or whole, broken, and groats. Red rice broken rice was physically damaged with sizes <6/10 to >2/10 of the whole grain, while groats had sizes <2/10 of the whole grain. The results showed that the head rice for A3 and A4 was about 69.7% and 55.6% lower than red rice without germination A1 and A2 at 82.63%. A5 and A6 increased by about 87.63% and 87.57%, while, broken rice A1 and A2 by 8.02%, A3 and A4 by 13.97% and 17.1%, while A5 and A6 decreased by 7.33% and 4.63%. Similarly, with rice groats, there was an increase in A3 and A4 (16.33 and 27.3%) compared to A1 and A2 (9.33%) and a decrease in germination A5 and A6 (5.03% and 7.8%), as shown in Table 2.

Table 3: Chemical properties of germinated red rice

Testing	Control (A1,A2)	UDRW (A3)	UDRC (A4)	RRW (A5)	RRC (A6)	INS 6128-2020[14]
Phosphorus(mg/100g)	136.59^{b}	105.08^{a}	172.63^{d}	142.82^{c}	238.71^{e}	-
Magnesium (mg/100g)	50.27^{c}	29.77^{a}	68.32^{d}	49.24^{b}	112.28^{e}	-
GABA (mg/kg)	21.97^{a}	144.08^{d}	231.96^{e}	86.31^{b}	132.88^{c}	-
Antioxidants IC_{50} (ppm)	676.07^{a}	1345.55^{c}	816.94^{b}	1892.89^{d}	636.04^{a}	-

Results of the Analysis of variance (ANOVA) showed that the interaction between the type of germination material and the type of soaking had a significant effect on the phosphorus, magnesium, GABA, and antioxidant activity of germinated red rice (Sig. 0.000) at the 5% level, so Duncan's further test was carried out, and it was found that there were significant differences. Based on the results in Table 3, it is known that the levels of phosphorus, magnesium, GABA, and antioxidant activity tend to increase in the germination treatment with the addition of chitosan (A4 and A6).

Discussion

Sensory Characteristics

Sensory characteristics based on panelists' level of preference for color, aroma, and texture parameters of germinated RR were analyzed using the hedonic method. The results showed that the variation of germination method had no significant effect on color, aroma, and texture parameters. Panelists' level of color preference ranged from 2.72 (neutral) to 3.37 (neutral), aroma parameter 2.99 (neutral) to 3.28 (neutral), and texture 2.95 (neutral) to 3.33 (neutral). In general, the process of germination and milling can reduce the color intensity of rice due to the nature of anthocyanin as an unstable color pigment less preferred by consumers.[27] The curing process can cause metabolic activity in the form of respiration increasing storage temperature, leading to the evaporation of volatile compounds such as hydrocarbons and alcohol.[13] The amylose and protein content also affect the hardness of rice. The higher starch content in the form of amylose fraction can cause the texture to become harder,[13,28] while the lower protein content reduces the hardness of rice.[29] This is in accordance with the results obtained that the lower protein and carbohydrate levels in germinated rice have a better preference level compared to treatments with higher protein and carbohydrate levels in the resulting treatment.

Milled Yield

Based on the results, germination using UDR tended to reduce milled yield, specifically with the use of chitosan (Control is 71.43%, UDRW is 70.07%, UDRC is 60.15%). Meanwhile, germination with RR produced a high total milled yield (Control is 71.43%, RRW is 87.21%, RRC is 85.8%). UDR germination reduced milled yield due to soaking process which caused the entry of water into the seeds and damaged the structure, hence, rice grains are easily broken during milling.[13] The more broken grains or groats in rice obtained can increase the weight loss due to the smaller particle size.[30]

Rice Quality

The decrease in quality observed in UDR germination was caused by the 48-hour soaking process which had a significant effect on rice grains produced.

Soaking and germination process can cause damage to rice cells, leading to a decrease in head rice yield after milling.[11] Meanwhile, germination on broken rice tends to increase because rice used has previously been milled to minimize the occurrence of cell damage. In terms of soaker type used, there was a more significant decrease in rice quality with chitosan. This is due to the hygroscopic nature which increases water content and causes damage to the cell structure.[30]

Color Characteristics

The color characteristics of RR germination from several germination methods were tested using a colorimeter tool. The results showed that the variation of germination method did not significantly affect the color characteristics. The L* value in A1 and A2 treatments amounted to 49.45, A3-A6 ranging from 48.41-52.34. In A1 and A2 treatments, a* value was 6.49, and in A3-A6 treatment, the value ranged from 3.3 to 5.37. Meanwhile, b* value in A1 and A2 treatments amounted to 10.91, and the value for A3-A6 ranged from 9.25-11.19. L* a* and b* values in germinated rice are influenced by the uncontrolled milling process because repeated milling can destroy the aleurone layer.[31] In addition, this can be caused by the nature of anthocyanins as color pigments that are unstable under certain conditions of temperature-sensitive properties, light, pH, oxygen, enzymes, chemical structure, and concentration of anthocyanins present.[27]

Morphology Structure

Morphological structure of germinated RR was observed using SEM (Fig. 1). The structure of samples A2 and A2 showed small cracks on the surface and the presence of starch grains. A3 showed sizable cracks on rice surface that are slightly smooth or absence of starch grains. A4 presented larger cracks on the surface with the presence of starch grains. Furthermore, sample A5 showed a chipped rice surface with a smoother surface without starch grains. In sample A6, the surface was rough, with large cracks and also the presence of starch grains. According to a previous study, germination process can induce cross-pattern sections, forming cracks, holes, pores, and messy starch structures.[32,33] Factors that can affect the formation of cracks in rice structure are soaking process and milling. Soaking process of UDR can cause the structure or cells in rice to be damaged due to the entry of water into rice.[13] Red rice tends to have more severe cracks than UDR germination. This is because, in germination process, the husk in RR has disappeared, leading to higher damage to rice structure. Chatchavanthatri *et al*. reported that germination process in RR can affect the morphology of starch grains compared to germination in grain.[34] Based on the results, using chitosan can damage the structure of rice due to the hygroscopic nature. The repeated milling process also increased the percentage of structural damage.

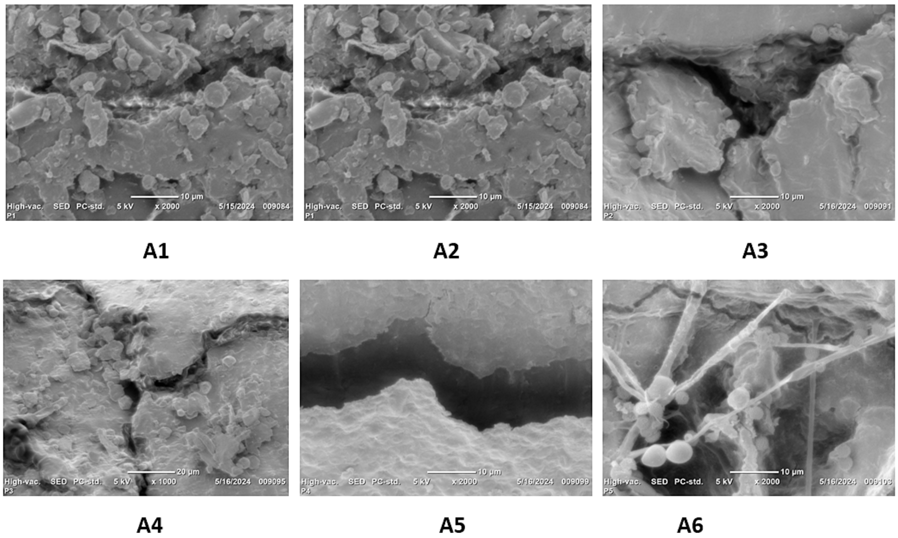

Fig. 1: Morphological structure of germinated red rice at 2000× magnification

Moisture Content

Water content of A1 and A2 was 10.73%, then the value increased in A3 and A6 to 11.53% and 11.17% respectively. Meanwhile, the value decreased in A4 by 8.63% and A5 by 9.33%. The type of germination material and soaking did not significantly affect the moisture content of rice but affected the interaction of the two factors. Based on the requirements of INS 6128-2020 the maximum moisture content in rice is 14%, hence, the results obtained are in accordance with the established standards. Factors that affect the value of moisture content during germination process include drying and soaking. Soaking process causes moisture content of a material to increase. The drying method also greatly affects moisture content of rice, by evaporating water in the material with heat energy. The temperature used greatly affects the quality of drying and can determine moisture content of rice.[35]

Ash Content

Ash content of A1 and A2 was 1.39%, while in germination treatment, the value decreased to 0.73% (A4), 0.84% (A6), 1.04% (A3), and 1.28% (A5). Both treatment factors and interaction did not significantly affect ash content. Aleuron is the inner layer of rice that stores minerals and iron.[35] The decrease in ash content of germinated RR was probably due to the rice grains are soaked in water to start the germination process. The ash content of the grains may decrease as a result of minerals and inorganic chemicals that contribute to the ash content leaking out into the soaking water.

Phosphorus

Phosphorus content in A1 and A2 was 136.59 mg/100 g then the value increased in A4, A5, and A6 to 172.63 mg/100 g, 142.82 mg/100 g, and 238.72 mg/100 g respectively. However, in A3, there was a decrease in phosphorus levels by 105.08 mg/100 g. Table 3 shows that phosphorus levels increased significantly due to the use of chitosan for germinating RR. The higher phosphorus levels were influenced by the use of chitosan as biostimulant.[36] Meanwhile, germination further increases phosphorus levels before milling process after germination which causes a decrease in mineral levels.[37] The increase in phosphorus content can be influenced during germination process which causes the breakdown of proteins. Generally, protein molecules consist of carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus. Minerals cannot be decomposed into simpler forms, but the decomposition of proteins can increase phosphorus levels.[38]

Magnesium

Magnesium content in A1 and A2 was 50.27 mg/100 g and increased in A4 and A6 to 68.32 mg/100g and 112.28 mg/100g respectively. However, in A3 and A5, there was a decrease in magnesium levels by 29.77 mg/100g and 49.24 mg/100g. The results showed that magnesium levels increased significantly in A4 and A6 due to the use of chitosan. Based on statistical tests, both types of treatment factors and interactions significantly affected the magnesium content of germinated RR. In general, RR acts as a source of magnesium, which is beneficial for health as it can manage type II diabetes, asthma, and bowel cancer.[1] Soaking for 48 hours allows a significant volume of water to enter the seeds, causing softening of the aleurone layer which contains many minerals. During milling process, the layer will be easier to escape and cause a decrease in magnesium content.[8,37] the use of chitosan in germination process increases magnesium in RR because its role as biostimulant.[36] The length of the grain or rice sprouts affects the increase in magnesium content in the germinated rice. The sprout length with the highest magnesium is about 1 cm at 37.0 mg/g.[39]

Protein Content

Protein content of A1 and A2 was 11.38%, while in germination treatment, the value decreased to 8.68% (A3), 9.99% (A4), 10.36% (A5), and 10.50% (A6). Both treatment factors and interactions did not significantly affect protein content. The decrease in protein content is due to the breakdown into amino acids, which are then used in the Krebs cycle (citric acid cycle). The energy obtained from the breakdown of protein is converted into ATP (Adenosine triphosphate). Furthermore, ATP formed acts as the main energy by cells in carrying out various biochemical functions including embryo growth.[40] The decrease in protein during germination is also influenced by the breakdown of protein, thereby increasing the soluble protein in rice.[41]

Gamma-Aminobutyric Acid (GABA)

The results in Table 3 show that A1 and A2 GABA levels amounted to 21.97 mg/kg, then variation in germination methods led to an increase in A3, A4, A5, and A6 to 144.08 mg/kg, 231.96 mg/kg, 86.31 mg/kg, and 132.88 mg/kg, respectively. The two treatment factors and interactions had a significant effect on GABA levels. Germination with UDR and soaking using an elicitor agent in the form of chitosan caused a significant increase in GABA from 21.97 mg/kg to 231.96 mg/kg. The increase during germination process is due to the activity of the enzyme glutamate decarboxylase (GAD) which converts glutamate into GABA. According to a previous report, GABA is released in response to to lack of water or oxygen, leading to increased activity of the enzyme GAD.[8] Chitosan can increase the activity of hydrolytic enzymes which function as food reserves for the embryo to improve the percentage of germination.[16,42] The increased activity of hydrolytic enzymes also enhances water absorption during the process. In addition, the increase in GABA levels is influenced by the variety of rice germination. This is due to the electron transfer between the O group of rice starch as well as the NH- and OH- groups of chitosan in forming hydroxyl groups.[12]

Fat Content

Fat content of A1 and A2 was 1.86%, while germination A3 increased by 4.07% but A4, A5, A6 treatments decreased by 1.85%, 1.34%, and 0.82% respectively. Both treatment factors and interactions significantly affected protein content. Germination tends to reduce fat content of rice due to the activity of lipase enzymes that break down fat into water-soluble fatty acids and glycerol. Therefore, more optimal germination can cause a decrease in fat content. The husk layer on rice during germination process tends to minimize the decrease in fat content. Chitosan, which can increase the activity of hydrolytic enzymes, is more optimal in germination process. As stated in a previous study, the decomposition of fat is higher than in water immersion treatment.[16,42] Grinding and drying processes reportedly reduced fat content.[43]

Carbohydrate Content

Carbohydrate content of A1 and A2 was 75.41%, while in germination A4, A5, A6, the value increased to 78.34%, 78.06%, 76.64% and decreased in A3 to 74.49%. Both treatment factors and interactions did not have a significant effect on carbohydrate content. During the initial germination process, carbohydrate content in rice generally decreases, which is influenced by the use as the main energy source and structural function.[44] However, germination process can also increase carbohydrate content because it is influenced by the duration. This increase is in line with the higher growth of rice grains.[45]

Antioxidant Activity

Antioxidant activity can be determined by calculating the capture of free radicals up to 50% using a compound present in the sample. The measurement is often carried out using UV-Vis spectrophotometry with a wavelength of 517 nm and expressed in IC_{50} (Inhibitory Concentration) value. Generally, IC_{50} is a value that states the concentration of antioxidant compounds present in the sample in counteracting free radicals by 50%. The higher the IC_{50} value, the lower the antioxidant activity of a sample.[46] Based on Table 3, IC_{50} of A1 and A2 was 676.07 ppm, while in germination A3, A4, and A5, the antioxidant activity decreased by 1345.55 ppm, 816.94 ppm, 1892.89 ppm, and increased in A6 by 636.04 ppm. Both treatment factors and interactions significantly affected antioxidant activity. Germination with RR and soaking using chitosan produced better antioxidant content. The increase in antioxidant levels during germination can be due to the response to environmental stress in the seeds. To overcome this stress, the plant will then increase the production of antioxidants as a self-defense mechanism from oxidative damage. The use of higher temperatures during the drying process can cause oxidation of antioxidant compounds due to the destruction of the chemical structure.[47] Heating can cause degradation of antioxidant compounds in the form of anthocyanins into ketones, thereby decreasing the ability of antioxidants to counteract free radicals.[48] Longer storage of food ingredients with conditions that are not optimal for food ingredients can reduce antioxidant activity due to oxidation.[49,50]

Conclusion

In conclusion, variations in germination methods significantly affected the physical and chemical characteristics of germinated RR. The results showed a significant increase in the levels of GABA, phosphorus, magnesium, milled yield, and rice quality. However, fat content, morphological structure, and antioxidant activity significantly decreased. There was no significant effect on sensory, carbohydrate, protein, ash, and moisture content. A5 had the highest yield of 87.21% and quality of 87.63%, while A4 had the highest GABA content of 231.96 mg/kg. And A6 had the highest antioxidant activity, phosphorus, and magnesium amounted to 636.04 ppm, 238.71 mg/100 g, and 112.28 mg/100 g respectively. However, conducting further research on applying different germination methods to white rice may yield varied results.

Acknowledgement

The authors would like to express high gratitude to the Institute of Research and Community Service of Hasanuddin University for providing research funding. Hasanuddin University, for permission to conduct research in the Food Science and Technology laboratory. The author also thanks all parties who contributed to the creation of this journal.

Funding Sources

Institute for Research and Community Service Hasanuddin University for the support of contract No. 00309/UN4.22/PT.01.03/2024.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

- **Andi Nur Faidah Rahman:** Writing-original draft, Visualization, Soft-ware, Methodology, Investigation, Approval of manuscript, Conceptualization.
- **Mulyati Tahir:** Conceptualization, Data curation, Format analysis, Methodology, Writing-original draft.
- **Jalaluddin Haddada:** Writing-review and editing, Format analysis, Validation.
- **Rasyidah Bakri:** Writing-review and editing, Validation, Data curation.
- **Amiruddin Hambali:** Methodology, Writing-review and editing, Validation.
- **Asmaul Husnah:** Writing-review and editing, Validation, Format Analysis.

References

1. Lee J-S., Sreenivasulu N., Hamilton R.S., Kohli A. Brown Rice, a Diet Rich in Health Promoting Properties. *J Nutr Sci Vitaminol.* 2019;65:26-28. DOI: 10.3177/jnsv.65.S26
2. Jati I.R.A.P., Darmoatmodjo L.M.Y.D., Suseno T.I.P., Ristiarini S., Wibowo C. Effect of Processing on Bioactive Compounds, Antioxidant Activity, Physicochemical, and Sensory Properties of Orange Sweet Potato, Red Rice, and Their Application for Flake Products. *Plants*. 2022;11(3):2-16. DOI:10.3390/plants11030440
3. Mohidem N.A., Hashim N., Shamsudin R., Man H.C. Rice for Food Security: Revisiting Its Production, Diversity, Rice Milling Process and Nutrient Content. *Agric*. 2022;12(6):1-28. DOI:10.3390/agriculture12060741
4. Amartani K. Germination Response of Corn Seed Germination (*Zea mays.* L) Under Salt Stress Conditions. AGROSAINSTEK: *J Agricultural Science and Technology.* 2019;3(1):9-14. DOI:10.33019/agrosainstek.v3i1.32
5. Finch-Savage W.E., Leubner-Metzger G. Seed Dormancy and the Control of Germination. *New Phytol.* 2006;171(3):501-523. DOI:10.1111/j.1469-8137.2006.01787.x
6. Permatasari L. Sprouts: A Potential Starch Hydrolyzing Agent. *Sasambo J Pharm.* 2022;3(2):111-114. DOI:10.29303/sjp.v3i2.174
7. Nirmagustina D.E., Wirawati C.U., Wienarto.

Physical and Chemical Characteristics of Germinated Brown Rice of 3 Types of Rice Varieties (Mentik Susu, Ciherang, Pandan Wangi). *J Gipas*. 2021;5(2):63-78. DOI: 10.20884/1.jgipas.2021.5.2.3898

8. Munarko H., Sitanggang A. B., Kusnandar F., Budijanto S. Germinated Brown Rice: Production Process and Its Characteristics. *J Pangan.* 2020;28(3):239-252. DOI: 10.33964/jp.v28i3.436
9. Setiati R., Siregar S., Wahyuningrum D., Fathaddin M.T. The Potential Success of Shrimp Shell as a Basic Material for Chitosan Polymer: Literature Study. Journal of Research and Scientific Works of the Trisakti University Research Institute. 2021;6(1):156-164. DOI:10.25105/pdk.v6i1.8635
10. Sivashankari P.R., Prabaharan M. 5 – Deacetylation Modification Techniques of Chitin and Chitosan. In Jennings J.A., Bumgardner J.D. Chitosan Based Biomaterials. Vol 1. *Elsevier;* 2017:117-133. DOI:10.1016/B978-0-08-100230-8.00005-4
11. Liu H., Zheng Z., Han X., Zhang C., Li H., Wu M. Chitosan Soaking Improves Seed Germination of Platycodon Grandiflorus and Enhances Its Growth, Photosynthesis, Resistance, Yield, and Quality. *Horticulturae.* 2022;8(10):1-13. DOI:10.3390/horticulturae8100943
12. Muhharam F.I. Chitosan Addition in Starch-Based Biofoam. *Edufortech*. 2020;5(2):119-127. DOI: 10.17509/edufortech.v5i2.28814
13. Rahman A.N.F., Asfar M., Suwandi N. The Effect of Grain Germination on Yield , Physical Quality, and Rice Sensory Value. *Indonesian Journal of Agricultural Postharvest Research*. 2020;17(3):177-183. DOI: 10.21082/jpasca.v17n3.2020.177-183
14. Indonesian National Standard. Rice. National Standardization Agency. Available online: https://uptdbpsmb.babelprov.go.id/sites/default/files/anggaran/SNI%206128%20 2020%20_%20Beras.pdf (accessed on August 2024).
15. Rachma Y.A., Anggraeni D.Y., Surja L.L., Susanti S., Pratama Y. Physical and Chemical Properties of Rough Red Rice Malt and Red Rice Malt Flour with Malting Treatment in Different Germination Time. *J Aplikasi Teknologi Pangan*. 2018;7(3):104-110. DOI:10.17728/jatp.2707
16. Maligan J.M., Lestary M., Wani Y.A. Differences in Antioxidant Activity of Brown Rice Sprouts (*Oryza sativa* L.) Based on Elicitation Process Duration and Germination Time*. Indones J Hum Nutr.* 2017; 4(2):108-116. DOI: 10.21776/ub.ijhn.2017.004.02.5
17. Firdaus M.J., Purwoko B., Dewi I.S., Willy S. Physicochemical Characterization of Dihaploid Black Rice Lines. *Indonesian J Agron*. 2022;50(1):1-9. DOI:10.24831/jai.v50i1.39850
18. Purbasari D. Application of Foam-Mat Drying Method in Processing of Instant Soybean Milk Powder. *J Agrotechnology.* 2019;13(01):52-61. DOI: 10.19184/j-agt.v13i01.9253
19. Dayani F., Sari R.N. Gluten-Free Cookies Formulation Using Local Composite Flour of Gadung, Brown Rice, and Moringa Leaves. *J Agritechnology Food Process.* 2023;3(1):1-13. DOI:10.31764/jafp.v3i1.15723
20. Setyaningsih N.E., Muttaqin R., Mar'ah I. Physics Communication Optimization of Gold-Palladium Coating Time on Natural Composite Materials for Morphological Characterization by Scanning Electron Microscopy (SEM)-Energy Dispersive X-Ray Spectroscopy (EDX). *Phys Comm*. 2017;1(2):36-40. http://journal.unnes.ac.id/nju/index.php/pc
21. Mohammed A., Abdullah A. Scanning Electron Microscopy (SEM): A review. Proc 2018 Int Conf Hydraul Pneum - HERVEX. 2019:77-85.
22. AOAC. AOAC: Official Methods 2011.14 Calcium, Copper, Iron, Magnesium, Manganese, Pottasium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Available online: https://pdfcoffee.com/201114-pdf-free.html (accessed on September 2024).
23. Corporation W. Acquity UPLC H-Class und H-Class Bio Amino Acid Analysis. Available online: https://www.waters.com/webassets/cms/support/docs/acq_uplc_h-class_aaa_sysgd_rev_b.pdf?srsltid=AfmBOorowJop_a13uyRsa3dH5Snc__Dg8SjLLOLzA4cO69kiOPdrBarm (accessed on August 2024).
24. Zieminska E., Toczylowska B., Diamandakis D., Hilgier W., Filipkowski R.K., Polowy R., Orzel J., Gorka M., Lazarewicz J.W. Glutamate, Glutamine and GABA Levels in

Rat Brain Measured Using MRS , HPLC and NMR Methods in Study of Two Models of Autism. *Front Mol Neurosci.* 2018;11:1-19. DOI:10.3389/fnmol.2018.00418

25. Piran F., Khoshkhoo Z., Hosseini S.E., Azizi M.H. Controlling the Antioxidant Activity of Green Tea Extract through Encapsulation in Chitosan-Citrate Nanogel. *J Food Qual.* 2020; 1-9. DOI:10.1155/2020/7935420
26. Kandi S., Linton A. Statistical Comparative Study Between The Conventional DPPH % Spectrophotometric and Dropping DPPH % Analytical Method Without Spectrophotometer: Evaluation for The Advancement of Antioxidant Activity Analysis. *Food Chem.* 2019;287:338-345. DOI:10.1016/j.foodchem.2019.02.110
27. Anggraeni V.J., Ramdanawati L., Ayuantika W. Determination Total Antocyanin in Brown Rice (Oryza nivara). Kartika: *J of Chemistry.* 2018;1(1):11-16. doi:10.26874/jkk.v1i1.11
28. Tao K., Yu W., Prakash S., Gilbert R.G. High-amylose rice: Starch Molecular Structural Features Controlling Cooked Rice Texture and Preference. *Carbohydr Polym.* 2019;219:251-260. DOI:10.1016/j.carbpol.2019.05.031
29. Benincasa P., Falcinelli B., Lutts S., Stagnari F., Galieni A. Sprouted Grains : A *Comprehensive Review*. 2019;11(2):1-29. DOI:10.3390/nu11020421
30. Rachmat R., Rahayu E., Darniadi S., Hadipernata M. Enhancement of Quality and Milled Rice Yield Recovery through the Improvement of Polishing Process and Configuration. *IOP Conf Ser Earth Environ Sci.* Published online 2022. DOI:10.1088/1755-1315/1024/1/012008
31. Nuryani. Potential Substitution of White Rice with Brown Rice. *Indonesian Community Nutrition Media.* 2013;3(3):157-168. https://journal.unhas.ac.id/index.php/mgmi/issue/download/279/39
32. Ren C., Hong B., Zheng X., Wang L., Zhang Y., Guan L., Yao X., Huang W., Zhou Y., Lu S. Improvement of Germinated Brown Rice Quality with Autoclaving Treatment. *Food Sci Nutr*. 2020;8(3):1709-1717. DOI:10.1002/fsn3.1459
33. Wang H., Xiao N., Ding J., Zhang Y., Liu X., Zhang H. Effect of Germination Temperature on Hierarchical Structures of Starch from Brown Rice and Their Relation to Pasting Properties. *Int J Biol Macromol*. 2020;147:965-972. DOI:10.1016/j.ijbiomac.2019.10.063
34. Chatchavanthatri N., Junyusen T., Moolkaew P., Arjharn W., Junyusen P. Effect of Soaking and Sprouting Treatment on Germination Rate of Paddy. E3S Web Conf. 2020;187:1-8. DOI:10.1051/e3sconf/202018704016
35. Hasnelly H., Fitriani E., Ayu S.P., Harvelly H. The Hulling Effect on Physical Quality and Nutritional Value of Different Types of Rice. *AgriTECH.* 2020;40(3):182-189. DOI: http://doi.org/10.22146/agritech.47487
36. Allam E., El-Darier S., Ghattass Z., Fakhry A., Elghobashy R.M. Application of Chitosan Nanopriming on Plant Growth and Secondary Matabolites of *Pancratium maritimum* L. *BMC Plant Biology*. 2024;24:1-13. DOI: 10.1186/s12870-024-05148-8
37. Tuncel N.Y., Andac A.E., Kaya H.P., Tuncel N.B. The Impact of Milling Degree on Physicochemical, Nutritional, Rheological, Textural Properties, and In Vitro Digestibility of Rice: From Brown Rice to White Rice. *Food Chem* 2025;464. DOI:10.1016/j.foodchem.2024.141795
38. Farooqui A.S., Syed H.M., Talpade N.N., Sontakke M.D., Ghatge P.U. Influence of Germination on Chemical and Nutritional Properties of Barley Flour. *J Pharmacogn Phytochem* 2018;7(2):3855-3858.
39. Rahman A.N.F., Asfar M., Suwandi N. The Effect of Grain Germination to Improve Rice Quality. *IOP Conf Ser Earth Environ Sci.* 2019;355(1):1-8. DOI:10.1088/1755-1315/355/1/012110
40. Henggu K.U., Nurdiansyah Y. Review of Carbohydrate, Lipid, Protein, and Nucleic Acid Metabolism. *Quimica.* 2022;3(2):9-17. DOI:10.33059/jq.v3i2.5688
41. Setiyoko A., Sari Y.P. The Improvement of Nutritional Characteristics and Antioxidant Potential of Cowpea Flour through Germination, Roasting and Its Combinations. *Agroindustrial Technol J.* 2023;7(3):19-37. DOI: 10.21111/atj.v7i3.10779
42. Stanley-Raja V., Senthil-Nathan S., Chanthini K.M-P., Sivanesh H., Ramasubramanian R., Karthi S., Shyam-Sundar N., Vasantha-Srinivasan P., Kalaivani K. Biological activity of Chitosan Inducing Resistance Efficiency

of Rice (Oryza sativa L.) after Treatment with Fungal Based Chitosan. *Sci Rep.* 2021;11(1):1-15. DOI:10.1038/s41598-021-99391-w

43. Sasmitaloka K.S., Widowati S., Sukasih E. Characterization of Physicochemical, Sensory, and Functional Properties of Instant Rice from Low Amylose Rice. *Indonesian Journal of Agricultural Postharvest Research.* 2020;17(1):1-14. DOI: 10.21082/jpasca.v17n1.2020.1-14
44. Nur A.M., Dwiloka B., Hintono A. The Effect of Germination Time on Physical Quality and Chemical Quality of Mucuna Pruriens Flour. *J Teknologi Pangan*. 2019;3(2):332-339. DOI: 10.14710/jtp.2019.24171
45. do Nascimento L.Á., Abhilasha A., Singh J., Elias M.C., Colussi R. Rice Germination and Its Impact on Technological and Nutritional Properties: A Review. *Rice Sci.* 2022;29(3):201-215. DOI:10.1016/j.rsci.2022.01.009
46. Artanti A.N., Lisnasari R. Antioxidant Activity Test of Ethanol Extract of Solanum Family Leaves Using Free Radical Reduction DPPH Method. JPSCR: *J Pharm Sci Clin Res.* 2018;3(2):62-69. DOI:10.20961/jpscr.v3i2.15378
47. Khasanah M.M., Ujianti R.M.D., Nurdyansyah F., Ferdiansyah M.K. Characteristics of Milkfish Crackers (Chanos chanos) from Variations in Fishmeal and Starch Processing Types. *J Postharvest and Marine and Fisheries Biotechnol.* 2020;15(2):143. DOI:10.15578/jpbkp.v15i2.634
48. Sugiyanto M.K., Sumual M.F., Djarkasi G. Effect of Pasteurization Temperature on Profile and Antioxidant Activity of Red Dragon Fruit Puree. *Agricultural Technol J.* 2020;11(2). DOI:10.35791/jteta.11.2.2020.31278
49. Prabowo A., Budhiyanti S.A., Husni A. Sargassum sp. Extract as an Antioxidant for Fish Oil Emulsion System During Storage at Room Temperature. *JPB Perikanan.* 2013;8(2):143-150. DOI: http://dx.doi.org/10.15578/jpbkp.v8i2.58
50. Nurhasnawati H., Sukarmi S., Handayani F. Comparison of Maceration and Soxhlet Extraction Methods on Antioxidant Activity of Guava Leaf Ethanol Extract (Syzygium malaccense L.). *J Ilmiah Manuntung.* 2017;3(1):91-95. DOI:10.51352/jim.v3i1.96