Germination of Six Indonesian Brown Rice: Evaluation of Antioxidant, Bioactive Compounds, Fatty Acids and Pasting Properties

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Keywords: Antioxidant, fatty acid, GABA, germinated brown rice, γ-oryzanol, pasting profiles

Posted Date: September 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-69330/v1

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Abstract

Background

Germination can improve the palatability and alter physicochemical, nutritional, and nutraceutical value of brown rice. This study aimed to evaluate the antioxidant, bioactive compounds, fatty acids, and pasting profiles from six Indonesian brown rice varieties during germination. The germination was carried out through a complete soaking method for up to 120 h, and the samples were taken every 24 h germination.

Results

The results showed that germination increased GABA content in brown rice. The highest level of GABA, up to 126.55 mg/100g, obtained in rice var. Inpari 43, after 120 h. Germination also affected the phenolic content, antioxidant capacity, and γ-oryzanol content, but no consistent trends were observed among the varieties. Fatty acid compositions of germinated brown rice showed no changes during germination. The pasting properties of samples changed significantly after germination, especially in peak viscosity, final viscosity, breakdown, and setback value.

Conclusion

In conclusion, the changes in brown rice characteristics during germination, especially for increased GABA content and shifting of pasting properties, are valuable information for developing functional rice-based food products.

Introduction

Rice is becoming one of the grain crops cultivated and consumed by the majority of people worldwide, especially in Asia and Africa region. It approximately contributes to half of the world population as a source of carbohydrate (Pengkumsri et al. 2015; Gong et al. 2017). Rice is considered a strategic commodity in Indonesia, not only as of the most primary food crop that cultivated by farmers but also as a staple food for about 240 million Indonesian people (Munarko et al. 2020).

Nowadays, increasing awareness to consume whole food as a healthy food has increased. Consuming whole food in regular diets has been associated with the reduced risk of chronic diseases, including cardiovascular disease, obesity, type II diabetes, and cancer (Gong et al. 2017). Brown rice or unpolished rice is considered as whole food because only the inedible outer layer part of the paddy is removed, while the bran layer still intact to the endosperm. A number of studies have shown that brown rice contains a high amount of antioxidant activity and bioactive compounds, including phenolic compounds, γ-oryzanol, and unsaturated fatty acid (Jayadeep and Malleshi 2011; Cáceres et al. 2017; Munarko et al. 2020). However, the existence of a bran layer in brown rice affects consumer preferences, especially on its harder texture, poor cooking, and eating quality (Xia et al. 2017; Munarko et al. 2019).

The germination process is able to improve the texture and taste of brown rice (Cho and Lim 2016). During germination, many hydrolytic enzymes such as α-amylase, β-amylase, and protease are activated. They hydrolyzed starch and protein resulted in an increase of oligosaccharides, sugars, and amino acids (Mohan et al. 2010). These changes also affected the physical characteristics, including its pasting properties of germinated brown rice (GBR) (Xu et al. 2012). Besides, germination also enhanced bioactive compounds such as γ-aminobutyric acid (GABA) (Ohtsubo et al. 2005; Zhang et al. 2014; Cáceres et al. 2017). GABA is a non-protein amino acid that has some essential physiological functions such as antihypertensive and anti-stress effects on human health (Iimure et al. 2009).
Commonly, the germination process may apply two procedures, i.e., a simple soaking method by steeping of the seeds in water, and temporary immersion followed by atmospheric germination (Cho and Lim 2016). The simple soaking method is the easiest procedure to produce GBR compared to the atmospheric germination. This method is suitably applied at the low production scale (individual or household scale), and in the areas where people easily access brown rice to produce GBR. In Indonesia, rice is commonly cultivated at local farmlands to cover their daily consumption. Therefore, it is easy to produce GBR at the household scale by applying the simple soaking method.

There are many rice varieties cultivated in Indonesia. In the last two decades, Indonesian Center for Rice Research has released many varieties that can be cultivated by farmers. Several varieties, such as Inpari 42, Inpari 43, Situ Bagendit, Inpari 17, and Inpara 3, are potentially high-yield varieties that have been developed. Inpari 42 and Inpari 43 are lowland irrigated rice cultivars that have low amylose content. Situ Bagendit is an upland rice variety with intermediate amylose content and is commonly cultivated by farmers in both dryland and paddy fields. Inpari 17 and Inpara 3 are categorized as high amylose rice and cultivated in lowland and swampland, respectively. Moreover, IPB University has released a new variety, namely IPB 3S. This variety includes lowland fields with intermediate amylose content (Munarko et al. 2020). Although many studies have reported the effect of germination on chemicals and bioactive compounds in many varieties over the world (Ohtsubo et al. 2005; Kiing et al. 2009; Zhang et al. 2014; Kaur et al. 2017), little is known especially for Indonesian rice varieties. Thus, this study aimed to evaluate the physicochemical and functional changes of six Indonesian brown rice varieties during germination.

**Materials And Methods**

**Materials**

Five rice varieties (Inpari 42, Inpari 43, Situ Bagendit, Inpari 17, and Inpara 3) were obtained from Indonesian Center for Rice Research (Subang, Indonesia), while IPB 3S variety was obtained from Seed Center, IPB University (Bogor, Indonesia). These rice varieties are certified paddy seeds. Ethanol, methanol, chloroform, 2-propanol, phenol, sodium hydroxide, folin-ciocalteu’s phenol reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, γ-oryzanol, and BF$_3$-methanol were purchased from Merck (Darmstadt, Germany). Fatty acid methyl esters C8-C22 (CRM 18920) and BHT were from Sigma Aldrich (USA). Boric acid was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). γ-aminobutyric acid (GABA) was from Acros Organics (New Jersey, US). Other chemical reagents used were analytical grade.

**Brown rice preparation**

Brown rice samples were prepared by the hulling process (Yanmar, Japan). The whole kernels were separated from broken rice using rice grader (Ogawa Seiki.Co.Ltd, Japan) and followed by manual sortation. Brown rice samples were vacuum sealed and stored at 4 °C for the further germination process.

**Germination procedure**

Germination procedure of brown rice followed the method of Ohtsubo et al. (2005) with a modification. Brown rice was sanitized using 0.1% sodium hypochlorite solution for 30 minutes and followed by rinsing in tap water. The sample was taken as 0 h treatment (ungerminated). The production of GBR was performed by soaking the sanitized brown rice in water (1:10, w/v) and incubated it in hot air sterilizer to maintain the water temperature at 33 ± 2 °C. The soaking water was removed and changed every four hours. The samples were removed after 24, 48, 72, 96, and 120 h and washed by using tap water before dried into a freeze dryer (Labconco, US). The dried GBR was ground using a blender (Miyako, Indonesia), sieved (40 mesh), vacuum sealed, and stored at −20 °C before analysis.
Evaluation of GABA (γ-aminobutyric acid) content

GABA content was analyzed duplicate according to the method of Zhang et al. (2014) with a slight modification in sample preparation. Sample (1.0 g) was placed in a plastic tube, and 5 mL deionized water was added. The mixture was then extracted for an hour, followed by a centrifugation at 3000 rpm for 30 minutes and filtered. A half milliliter of supernatant was added with 0.2 mL borate buffer (pH 9), 1.0 mL phenol reagent 6% (w/v), and 0.4 mL sodium hypochlorite 9% and then mixed vigorously. The tube was then boiled in the water bath for 10 minutes and cooled immediately in ice water for 20 minutes to develop a blue color. The absorbance was measured by a UV-Vis spectrophotometer (Thermo Scientific 150, US) at 645 nm. Standard curve calibration of GABA was prepared to determine GABA concentration in the samples and expressed as mg GABA/100 g dried samples.

Determination of total phenolic content and antioxidant capacity

Phenolic compounds were extracted using ethanol extraction (Munarko et al. 2020). The rice flour (approximately 1.5 g) was extracted with 20 mL of ethanol 80% (v/v) using a shaker (Innova2300, new Brunswick scientific) for 30 minutes and centrifuged at 6000 rpm (Hermle Z 383 K, Wehingen, Germany) at 4 °C for 30 minutes. The supernatant was collected into a dark bottle and stored at 4 °C.

Total phenolic content (TPC) was analyzed duplicate by the modified Folin-Ciocalteau method (Qiu et al. 2010). Extract (0.2 mL) was mixed with 1.8 mL of 10 × diluted Folin-Ciocalteau reagent (freshly prepared) and 1.8 mL of Na₂CO₃ 60 g/L. After reacting for 90 minutes, the mixture was analyzed by a UV-Vis spectrophotometer (Thermo Scientific 150, US) at 725 nm. The absorbance was compared to the standard curve of gallic acid and expressed as mg gallic acid equivalent per 100 g dry samples (mg GAE/100 g).

Determination of DPPH scavenging activity was measured duplicate by the method originally developed by Brand-Williams et al. (1995) with a modification. The phenolic extract of rice (0.3 mL) and 0.7 mL of distilled water were mixed with 3.0 mL of freshly DPPH solution 140 µM and incubated in the darkroom for 60 minutes. The mixture was then analyzed using a UV-Vis spectrophotometer (Thermo Scientific 150, US) at 515 nm. The antioxidant activity was expressed by mg ascorbic acid equivalent per 100 g of dried sample (mg AAE/100 g).

Analysis of γ-oryzanol

Analysis of γ-oryzanol applied a partial extraction method (Lilitchan et al. 2008). Two identical rice flour samples (1.0 g) were extracted with isopropanol using different volumes (4 mL and 8 mL) and centrifuged for 10 minutes at 2500 rpm (Eppendorf 5810R). The absorbance of the supernatant was measured using a UV-Vis spectrophotometer (Thermo Scientific 150, US) at 326 nm. Total γ-oryzanol of the sample was compared to the γ-oryzanol standard curve and then calculated as follows (Eq. 1):

$$y = \frac{x_1 x_2}{2 x_1 - x_2} \quad (1)$$

Where y is the concentration of γ-oryzanol in the rice samples (expressed as mg/100g dry sample), \(x_1\) is the concentration of γ-oryzanol in 4 mL extract, and \(x_2\) is the concentration of γ-oryzanol in 8 mL extract.

Analysis of fatty acid composition

Lipid extraction applied the method of Bligh and Dyer (1959) with a modification (Munarko et al. 2020). Sample (5.0 g) was mixed with distilled water, chloroform, and methanol to reach chloroform:methanol:water became 1:2:0.8 (v/v/v),
followed by centrifugation at 4000 rpm (Hermle Z 383 K, Wehingen, Germany) for 10 minutes. The sample was added chloroform and water containing 0.85% of KCl to reach the final ratio of 2:2:1.8 of chloroform:methanol:water (v/v/v). The mixture was centrifuged at 4000 rpm (Hermle Z 383 K, Wehingen, Germany) for 10 minutes and then filtered to remove the solid. The supernatant was allowed to separate into two phases. The lower chloroform phase was collected and evaporated using nitrogen gas at 50 °C.

Fatty acid derivatization was prepared by using the BF$_3$ methanol catalyst, and the analysis of fatty acid composition was assayed by Gas Chromatography 7890A (Agilent Technologies, California, US) with a flame ionization detector (FID). The column was DB-23 (J and W Scientific, Folsom, CA) with a dimension of 60 m × 0.25 mm, film thickness of 0.25 µm. The temperature of the detector and injector was set at 280 °C and 270 °C, respectively. The initial temperature was set at 130 °C and held for 2 min. It increased to 170 °C at a rate of 6.5 °C/min and held for 5 min. Then, it increased to 215 °C at 2.75 °C/min and held for 12 min, followed by increased to 230 °C at 30 °C/min and held for 30 min. Helium and nitrogen from ultra-high purity grade at flow rate 11.07 and 31.24 mL/min, respectively, were used as carrier gasses. Identification of fatty acid composition was determined by comparing the retention time of each peak in the sample containing internal standard (C17:0) with the respective external standard (C8:0 to C22:0) containing internal standard of fatty acid methyl esters (Taufik et al. 2016).

Analysis of pasting profile

Pasting profiles of brown rice flour were carried out duplicate by using a rapid visco analyzer (RVA) (Tec-Master, Newport Scientific, Australia). Sample (3.0 g, moisture content 14%) was diluted in 25 g of aquadest, followed by heating and cooling cycle with constant stirring. The sample was heated at 50 °C for 1 min in advance of heating to 95 °C at 6 °C/min and maintained for 5 min. The temperature reduced to 50 °C at 6 °C/min and then held at 50 °C for 5 min.

Statistical analysis

Analysis of the variance and significance of the differences among the samples were conducted by analysis of variance (ANOVA) procedure and Duncan’s multiple range test of SPSS software version 22.

Results And Discussion

The effect of germination on GABA content

The changes in GABA content during germination are presented in Fig. 1. GABA contents in brown rice relatively low, ranging from 19–27 mg/100 g. The accumulation of GABA increased significantly during germination. GABA content in GBR var. Inpari 42, Inpari 43, and IPB 3S increased after soaked for 24 h. However, decreasing in GABA content was observed in var. Inpari 17 and Inpara 3. At 48 h and 72 h of soaking time, all varieties showed increased GABA content. The highest GABA content at 72 h treatment was found in var. Inpari 43 (85.11 ± 0.71 mg/100 g), followed by IPB 3S, Situ Bagendit, Inpari 42, Inpari 17, and Inpara 3 (31.17 ± 3.57 mg/100 g). At the longer soaking time, five of six varieties showed the highest level of GABA content. The highest GABA content observed after soaking at 120 h in var. Inpari 43 (126.55 ± 2.54 mg/100 g), followed by var. IPB 3S (98.80 ± 1.48 mg/100 g), Situ Bagendit (88.23 ± 0.52 mg/100 g), Inpari 42 (81.66 ± 4.60 mg/100 g), and Inpari 17 (75.37 ± 0.76 mg/100 g). Meanwhile, a different trend was reported in GBR from var. Inpara 3 which decreased consistently during germination for 96 h and 120 h.

The increase of GABA content during germination was reported in seeds and legumes, including in brown rice (Mohan et al. 2010; Zhang et al. 2014; Tiansawang et al. 2016). Our study showed that germination was able to increase the
accumulation of GABA in all varieties. The increase of GABA content in germinated brown rice from six varieties ranged from 1.6 (var. Inpara 3) to 4.7 times (var. Inpari 43). The results were comparable to that of brown rice var. KDML 105 and CNT 1 which increased the GABA content up to 73.05 mg/100 g and 92.42 mg/100 g after soaking for 72 h (Kaosa-ard and Songsermpong 2012). The accumulation of GABA in germinated brown rice var. Koshihikari also increased up to 149.03 mg/100 g after germination for 96 h (Ohtsubo et al. 2005).

GABA content in germinated brown rice varied among rice varieties. These results are in-line with previous studies in several varieties from different geographical regions. GABA content in germinated brown rice var. Indica Guichao was reported higher than in var. Japonica Jing 305 (Zhang et al. 2014). Four Ecuadorian sprouted brown rice showed significant differences after soaking and incubating at designed conditions (Cáceres et al. 2014). GBR from ten cultivars in India also exhibited significantly different among the samples (Kaur et al. 2017).

The increase of GABA during germination was closely related to the activation of some enzymes that converts glutamate to succinate via GABA, called GABA shunt. The first step applies the direct and irreversible α-decarboxylation of glutamate by glutamate decarboxylase (GAD enzyme). The second enzyme is GABA transaminase (GABA-T) which catalyzes the reversible conversion of GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate as amino receptors. The last step of the GABA shunt is catalyzed by succinic semialdehyde dehydrogenase (SSADH), which is irreversible oxidizing succinic semialdehyde to succinate (Shelp et al. 1999). Increasing GABA content was related to the increasing activity of the GAD enzyme. According to Zhang et al. (2014), GAD enzyme activity increased during germination in brown rice var. Guichao 2 and Jing 305. At the longer germination time, GAD activity decreased and followed by decreasing GABA content. In the case of GABA content in var. Inpara 3 from our research, the decrease of GABA content after 96 h and 120 h germination might be related to the decrease of GAD activity; thus the rate of GABA production was lower than the conversion of GABA to succinate.

**The effect of germination on total phenolic content and antioxidant capacity**

Figure 2 shows TPC and antioxidant capacity of six Indonesian brown rice during germination. Germination of brown rice affected the TPC and antioxidant activity. After 24 h steeping time, both TPC and antioxidant activity of six germinated brown rice decreased significantly. The results are agreed with the results in brown rice Indica SLF09 and some different grains after soaking for 24 h and 6 h, respectively (Tiansawang et al. 2016; Cáceres et al. 2017). During the soaking process and changing the soaking water, some phenolic compounds might leach out to the water and affect the reduction of phenolic content in GBR.

At the longer period of soaking time, there were some different trends found in each variety of GBR. In Inpari 42, antioxidant activity showed no significant changes for the longer steeping time, and TPC slightly changed. However, the amount of TPC and antioxidant activity in all periods of germination time was lower than in ungerminated brown rice. Brown rice var. Inpari 43, Situ Bagendit, IPB 3S, and Inpari 17 represented similar trends after the soaking process. Both of antioxidant capacity, as well as TPC, were increasing after soaked at 48 to 96 h compared to that of 24 h. In contrast, TPC and antioxidant capacity of GBR from var. Inpara 3 decreased for the longer progress of soaking time. The results are agreed with Phattayakorn et al. (2016), who observed in three different varieties during germination. The TPC of red non-waxy rice (red Jasmine) variety decreased significantly, although the DPPH scavenging activity did not change after germination. In contrast, black waxy rice and white non-waxy rice (KDML105) increased both of total phenolic content and the antioxidant activity. From this study, it is important to pay attention that the technological properties applied in the germination process might affect the accumulation of TPC and antioxidant activity in GBR.

**The effect of germination on γ-oryzanol content**
The γ-oryzanol is a bioactive compound in the lipophilic fraction that be the major substance found in brown rice. It consists of ten or more compounds with ester bonds between ferulic acid and triterpenes (Cho and Lim 2016). γ-oryzanol content in brown rice during germination is shown in Table 1. The highest γ-oryzanol content of ungerminated brown rice was found in var. Situ Bagendit (57.62 ± 2.72 mg/100 g) and var. Inpara 3 (57.40 ± 2.12 mg/100 g). The γ-oryzanol content from var. Inpari 42 and var. Inpari 17 did not change after soaking for 24 h, but they decreased after the longer soaking time. In var. IPB 3S, the γ-oryzanol content did not change notably during 48 h of germination. It decreased slightly at 72 and 96 h and then increased up to the highest level after germination for 120 h. Moreover, the γ-oryzanol content in Inpari 43 slightly increased after soaking for 24 h; even it decreased afterward.

| Varieties          | Soaking times (h) | 0  | 24  | 48  | 72  | 96  | 120 |
|--------------------|-------------------|----|-----|-----|-----|-----|-----|
|                    |                   |    |     |     |     |     |     |
| Inpari 42          |                   |    |     |     |     |     |     |
|                    |                   | 41.98 ± 0.71a | 41.98 ± 0.71a | 38.53 ± 0.39b | 37.33 ± 0.58c | 38.45 ± 0.85b | 36.96 ± 1.70c |
| Inpari 43          |                   |    |     |     |     |     |     |
|                    |                   | 39.10 ± 0.79b | 42.37 ± 0.84a | 37.37 ± 2.09c | 36.26 ± 1.70d | 34.42 ± 1.06e | 38.25 ± 0.29bc |
| Situ Bagendit      |                   |    |     |     |     |     |     |
|                    |                   | 57.62 ± 2.72a | 49.95 ± 2.27b | 47.70 ± 0.44c | 48.02 ± 0.90c | 45.09 ± 1.01d | 47.71 ± 2.05c |
| IPB 3S             |                   |    |     |     |     |     |     |
|                    |                   | 44.44 ± 0.47b | 44.40 ± 1.22b | 44.34 ± 1.39b | 38.55 ± 0.69c | 38.99 ± 2.36c | 47.45 ± 1.13a |
| Inpari 17          |                   |    |     |     |     |     |     |
|                    |                   | 47.81 ± 1.21a | 48.05 ± 1.49a | 43.66 ± 1.65bc | 42.92 ± 1.11c | 43.49 ± 1.69bc | 44.92 ± 1.91b |
| Inpara 3           |                   |    |     |     |     |     |     |
|                    |                   | 57.40 ± 2.12a | 50.69 ± 0.93b | 47.99 ± 2.30c | 44.91 ± 1.40d | 44.03 ± 2.30d | 43.81 ± 2.27d |

Note: Values with different letters in the same row are significantly different (p < 0.05)

Changes of γ-oryzanol in GBR were still unclear, either in increasing or decreasing during germination. Some studies reported in decreasing γ-oryzanol content and some of them showed increasing or not significant changes. GBR from var. indica SLF09 decreased by approximately 15% after soaking for 24 h (Cáceres et al. 2017). The γ-oryzanol of five of eight brown rice varieties from South Sarawak, Malaysia also exhibited a slight decrease after germination by soaking for 24 h (Kiing et al. 2009). The Japanese brown rice (Koshihikari) soaked for 72 h at 30 °C showed at the same level in γ-oryzanol content compared to the ungerminated brown rice (Ohtsubo et al. 2005). The study about germination in four Korean rice varieties (Ilpum, Gaomi2, Keunnun, and Heugkwang) reported that germinated brown rice var. Keunnun and Heugkwang had higher γ-oryzanol compared to the ungerminated brown rice, whereas the others remained unchanged (Lee et al. 2007).

**Fatty acid profiles of germinated brown rice**

The fatty acid composition was analyzed for ungerminated brown rice and selected GBR at 120 h germination (Table 2). The germination process did not affect the composition of fatty acids as indicated by the identical fatty acids compositions in ungerminated brown rice and GBR. Both in ungerminated brown rice and GBR 120 h, the composition of fatty acids was dominated by palmitic acid (C16:0), oleic acid (C18:1 cis), and linoleic acid (C18:2),
which contributed to 17–19%, 33–36%, and 37–41% of the fatty acid composition, respectively. The remaining minor compounds of fatty acids consisted of myristic (C14:0), palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3), arachidic acid (C20:0), cis-11 eicosenoic (C20:1), docosanoic acid (C22:0). Based on its saturation, both ungerminated brown rice and GBR were dominated by unsaturated fatty acids that contributed to 77–79% of total fatty acid content, whereas saturated fatty acids only contributed approximately 21–23%. These results were comparable to the study of Jayadeep and Malleshi (2011) in brown rice var. IR 64 and BPT that reported no obvious changes in fatty acid compositions after germination.

| Fatty acids | Inpari 42 BR | Inpari 43 BR | Situ Bagendit BR | IPB 3S BR | Inpari 17 BR | Inpara 3 BR | GBR BR | GBR GBR | GBR BR | GBR GBR |
|------------|--------------|--------------|------------------|---------|--------------|--------------|---------|---------|---------|---------|
| C14:0      | 0.57         | 0.82         | 0.67             | 0.73    | 0.56         | 0.70         | 0.74    | 0.97    | 0.75    | 0.58    | 0.70    | 0.84 |
| C16:0      | 18.31        | 17.98        | 18.85            | 17.51   | 17.51        | 17.25        | 18.37   | 19.25   | 18.25   | 18.19   | 19.46   | 19.73|
| C16:1      | 0.20         | 0.23         | 0.19             | 0.18    | 0.17         | 0.21         | 0.17    | 0.17    | 0.21    | 0.19    | 0.19    | 0.21|
| C18:0      | 1.62         | 1.74         | 1.75             | 1.65    | 1.61         | 1.74         | 1.73    | 1.79    | 1.64    | 1.91    | 2.04    | 2.17|
| C18:1cis   | 35.92        | 36.00        | 35.86            | 34.41   | 34.60        | 35.36        | 34.39   | 33.63   | 35.83   | 34.36   | 34.71   | 34.96|
| C18:2      | 40.32        | 40.12        | 38.88            | 40.52   | 40.90        | 41.34        | 41.19   | 39.62   | 40.26   | 41.56   | 37.49   | 37.39|
| C18:3      | 2.02         | 2.03         | 2.36             | 2.88    | 2.66         | 2.31         | 2.34    | 2.84    | 2.00    | 2.08    | 3.47    | 3.10|
| C20:0      | 0.29         | 0.30         | 0.37             | 0.36    | 0.36         | 0.29         | 0.30    | 0.29    | 0.29    | 0.38    | 0.34    | 0.31|
| C20:1      | 0.41         | 0.47         | 0.56             | 1.18    | 1.00         | 0.44         | 0.46    | 0.88    | 0.42    | 0.41    | 0.83    | 1.02|
| C22:0      | 0.34         | 0.31         | 0.50             | 0.57    | 0.62         | 0.29         | 0.32    | 0.56    | 0.34    | 0.36    | 0.77    | 0.27|
| SFA        | 21.13        | 21.15        | 22.14            | 20.82   | 20.67        | 20.34        | 21.45   | 22.86   | 21.28   | 21.41   | 23.31   | 23.32|
| USFA       | 78.87        | 78.85        | 77.86            | 79.18   | 79.33        | 79.66        | 78.55   | 77.14   | 78.72   | 78.59   | 76.69   | 76.68|
| TFA        | 100          | 100          | 100              | 100     | 100          | 100          | 100     | 100     | 100     | 100     | 100     | 100 |

Note: BR = Brown rice; GBR = Germinated brown rice (at 120 h); SFA = Saturated fatty acids; USFA = Unsaturated fatty acids; TFA = Total fatty acids; All numbers are presented in % (mg/100 mg total fatty acid)

List of Figures

The effect of germination on pasting profiles of germinated brown rice

The pasting profiles of six Indonesian brown rice varieties during germination was presented in Fig. 3. The germination process significantly modified the pasting profiles of brown rice for all rice varieties. In this study, germination of brown rice by the soaking method considerably impact the reduction of peak viscosity, trough viscosity, breakdown, setback, and final viscosity. The lowered peak viscosity occurred especially after soaking for 48 h or longer.

The pasting behavior of food materials during the heating and cooling process influences the quality of final the products (Xu et al. 2012). The GBR in all varieties experienced the decline of peak viscosity, trough viscosity, breakdown, setback, and final viscosity. The decrease of peak viscosity was attributed to the presence of endogenous
hydrolytic enzymes activity such as amylase enzyme, which hydrolyzed starch to smaller molecules (Wichamanee and Teerarat 2012; Wu et al. 2013). It is well documented in the previous study that α-amylase, as well as β-amylase enzymes, increased as germination progressing, thus, leading to a decrease in peak viscosity (Mohan et al. 2010; Pinkaew et al. 2016). Germination also reduced trough and breakdown viscosity which related to the stability of the flour during the heating process (Mohan et al. 2010). After heating at a particular time, the pasting flour was then cooled down to obtain the final viscosity and setback value. The setback and final viscosity usually represent the retrogradation tendency. In this study, the reduction of setback value indicates that GBR is more stable against retrogradation (Yuliana and Akhbar 2020).

**Conclusion**

The germination process from six Indonesian rice varieties by a simple soaking method, was found to significantly increase the GABA content of all Indonesian varieties used in this study. The highest GABA content was obtained in GBR var. Inpari 43 which increased consistently up to 4.7-fold after soaking 120 h. The lowest trend of GABA content found in var. In part 3 that increased 1.6-fold after 72 h soaking. TPC, antioxidant activity, and γ-oryzanol contents did not increase after germination, as well as fatty acid compositions. Germination influenced the pasting profiles of GBR. The longer period of soaking time caused the lower peak viscosity, breakdown, and setback value. Based on this, GBR was less viscous and more stable during heating and cooling.

**Abbreviations**

GABA  
γ-aminobutyric acid  
TPC  
total phenolic content  
GBR  
germinated brown rice  
DPPH  
2,2-Diphenyl-1-picrylhydrazyl  
GAD  
glutamate decarboxylase  
GABA-T  
GABA transaminase

**Declarations**

*Ethics approval and consent to participate*

Not applicable

*Consent for publication*

Not applicable

*Availability of data and materials*

All data generated or analysed during this study are included in this published article.
Competing interests

The authors declare that they have no competing interests

Funding

This research was funded by The Ministry of Education and Culture in *Pendidikan Magister menuju Doktor untuk Sarjana Unggul* (PMDSU) scholarship program for the first author.

Authors’ contributions

HM: methodology, investigation, formal analysis, writing - original draft; ABS: conceptualization, methodology, writing - original draft, funding acquisition; FK: conceptualization, writing - review & editing; SB: conceptualization, writing - review & editing, funding acquisition, supervision; All authors read and approved the final manuscript

Acknowledgements

The authors would like to acknowledge The Ministry of Research and Technology, and The Ministry of Education and Culture for supporting this research by *Pendidikan Magister menuju Doktor untuk Sarjana Unggul* (PMDSU) scholarship program for the first author.

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Figures

Figure 1

GABA profiles of germinated brown rice from six Indonesian rice varieties at different steeping times.
Figure 2

Total phenolic content (TPC) and antioxidant activity (AA) of germinated brown rice from six Indonesian rice varieties (bar chart for DPPH scavenging activity; line chart for TPC). A = Inpari 42, B = Inpari 43, C = Situ Bagendit, D = IPB 3S, E = Inpari 17, F = Inpara 3. Different letters indicate values are significantly different (p<0.05).
Figure 3

Pasting properties changes of brown rice and germinated brown rice: A = Inpari 42, B = Inpari 43, C = Situ Bagendit, D = IPB 3S, E = Inpari 17, F = Inpara 3.

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