TiS Trends in Sciences
TRENDS IN SCIENCES 2027; 24(1): 14366
SPRINT Scholarly Publishing and Repository Integration Center
https://doi.org/10.48048/tis.2027.14366
RESEARCH ARTICLE

Germination-Induced Changes in Fatty Acid, Amino Acid, and Bioactive Compound Profiles of Indonesian IR-64 Brown Rice and Cempo Ireng Black Rice

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Received: 11 May 2026, Revised: 29 June 2026, Accepted: 9 July 2026, Published: 20 August 2026

Abstract

Germination has been recognized as an efficient and effective technique for enhancing the nutritional content and bioactive compounds in a variety of cereal products. Several studies have reported that germination in rice can increase the levels of various compounds. However, changes induced by germination in Indonesian rice varieties are still rarely reported. Germinated IR-64 brown rice and Cempo Ireng black rice have demonstrated various significant roles based on *in vivo* studies, but the specific compounds responsible for these effects remain insufficiently characterized and their changes during germination have not been clearly reported. These rice varieties possess substantial potential for development as functional food products owing to their important roles in human health. Therefore, to address this research gap, changes in bioactive compounds as well as fatty acid and amino acid profiles during germination in IR-64 brown rice and Cempo Ireng black rice were analyzed, employing HPLC, spectrophotometry, and GC. After 48 h of germination at 33 °C, consisting of 24 h of soaking followed by 24 h of aeration, changes were observed in fatty acids, amino acids, GABA, γ-oryzanol, vitamin E, and vitamin K. Increases in fatty acids, GABA, and γ-oryzanol were detected in both rice varieties, whereas, vitamin K1 (phylloquinone) exhibited a 2-fold increase only in IR-64 brown rice. The total amino acid content in both varieties remained relatively stable, indicating that germination did not negatively affect the protein-related nutritional quality of the rice. In contrast, vitamin E (tocopherol) decreased after germination, possibly due to its role as an antioxidant during the germination process. These results highlight the significance of germination in modifying the biochemical composition of various rice varieties and indicate its potential as a method to enhance nutritional quality for the development of functional food products.

Keywords: Germination, Brown rice, Black rice, IR-64, Cempo ireng, Bioactive compounds

Introduction

The majority of Indonesians rely on rice as their main staple food [1]. In line with this high consumption, rice production in Indonesia is also abundant. It has been reported that Indonesia possesses approximately 257 rice varieties, with annual rice production reaching 53.98 million tons, making Indonesia the third largest rice producer in the world after India and China [1,2]. The availability and diversity of rice in Indonesia therefore provide significant potential for further research and development.

Unpolished rice is a type of rice that undergoes incomplete milling, in which only the husk is removed while the bran layer remains intact, resulting in brown, red, or black rice, determined by the color of the bran layer [3–5]. Due to the presence of this bran layer, unpolished rice provides various levels of nutrients and

bioactive compounds, including lipids, proteins, oryzanol, dietary fiber, vitamins, minerals, and tocopherols [6]. By contrast, polished rice is obtained after more extensive milling and polishing, which eliminates both the husk and bran layers, and produces white rice [4]. As a consequence of this removal process, the nutritional content and bioactive compounds in polished rice are significantly reduced [7,8]. However, despite the higher nutritional value of unpolished rice, its consumption remains relatively low due to its harder and rougher texture [9]. Previous studies have proposed germination as a potential solution to improve this limitation [10].

Germination or sprouting is considered one of the simplest and most efficient processes to improve the texture of unpolished rice without compromising its nutritional value and bioactive compounds [11]. It has been reported that germination affects the profiles of fatty acids, amino acids, GABA (γ-aminobutyric acid), γ-oryzanol, and vitamins in unpolished rice. Interestingly, in Fortuna-type and Minghui 86 rice, polyunsaturated fatty acids and free fatty acids, respectively, have been reported to increase following germination [12,13]. Similarly, in NERICA-8 and FARO 44 rice, several amino acids have been shown to increase after germination [14,15]. GABA levels have also been reported to increase as a result of germination in nearly all studied rice varieties, including Hom Nil, Niew Dam, Situ Bagendit, Inpari 17, Japonica-type, FARO 44, and NERICA-8 [10,14–17]. In addition, γ-oryzanol content has been reported to increase after germination in Buebang 3 Morchor and RD-8 rice [18,19], and a similar trend has been observed for vitamin E, which increases following germination in Buebang 3 Morchor, NERICA-8, and FARO 44 rice [14,15,18]. These compounds play important roles in promoting human health, including improving cardiovascular health, reducing the risk of metabolic syndrome, enhancing pancreatic β-cell viability, and exhibiting anti-inflammatory effects [20–25].

However, studies investigating the profiles of fatty acids, amino acids, GABA, γ-oryzanol, as well as vitamins E and K during germination of Indonesian rice varieties remain very limited. Among the numerous rice varieties available in Indonesia, this study focuses on the IR-64 and Cempo Ireng varieties due to their high availability [26]. IR-64 is a widely consumed white rice variety because of its affordability and desirable quality, whereas Cempo Ireng is a local black rice variety originating from Yogyakarta [27,28]. Previous *in vivo* studies have reported that germinated IR-64 rice can suppress the elevation of blood glucose levels, support pancreatic β-cell regeneration, and lower total cholesterol [29,30]. Similarly, germinated Cempo Ireng has been reported to improve obesity-related conditions, while rice bran derived from the Cempo Ireng variety has been reported to exhibit anticancer potential against T47D breast cancer cells [31,32]. Despite these findings, the specific compounds responsible for these functional properties have not yet been clearly identified, and their changes during germination remain unreported.

Understanding how germination affects these compounds in these two rice varieties is important, considering their potential for functional food development and their relevance to human health. Accordingly, this study aimed to identify germination-induced changes in the fatty acids and amino acids profiles, GABA, γ-oryzanol, vitamins E, and vitamin K of IR-64 brown rice and Cempo Ireng black rice. The results of this study are expected to serve useful scientific information for further research and applications in the development of functional foods, as well as for improving human health.

Materials and methods

Materials

IR-64 paddy was purchased from Balai Benih Pusaka Jaya, West Java, Indonesia, while Cempo Ireng paddy was obtained from PT Berkat Agro Jaya, Yogyakarta, Indonesia. The chemicals used included chloroform, methanol, sodium acetate, tetrahydrofuran (THF), ethanol, *n*-hexane, distilled water, sodium hypochlorite, BF_3-methanol, hydrochloric acid, sodium hydroxide, *o*-phthaldialdehyde, 2-mercaptoethanol, boric acid, potassium chloride, acetonitrile, and diethyl ether. All reagents were of analytical grade (Merck, Germany). The reference standards included FAME Mix C8–C24 (Merck, Germany), amino acid standard mixture (Sigma-Aldrich, USA), GABA (γ-aminobutyric acid) (Merck, Germany), γ-oryzanol (ChromaDex, USA), vitamin E (tocopherol) (Sigma-Aldrich, USA), and vitamin K (phylloquinone, menaquinone-3, and menaquinone-4) (Eisai, Japan).

Sample preparation

IR-64 brown rice and Cempo Ireng black rice were prepared by milling dried paddy using a milling machine (Yasuka, China). Husk removal and sorting were carried out manually. After being packaged under vacuum conditions, the samples were stored at room temperature (27 °C) until further processing [16].

Germination procedure

The process of germination was conducted utilizing a synergistic approach that incorporated both soaking and aeration techniques. Initially, the rice was immersed in a 0.1% sodium hypochlorite solution for a duration of 30 min, followed by a thorough rinsing with distilled water. Samples representing the non-germinated were collected at this stage. Germination was initiated by placing the rice in a sterile container, adding distilled water at a ratio of 1:3 (w/v), and then soaking the rice at 33 °C. Every 8 h, the soaking water was replaced with fresh distilled water. After 24 h of soaking, the samples were transferred to sterile containers for aeration at 33 °C. During the aeration stage, distilled water was sprayed every 8 for 24 h [16]. Rice grains that failed to germinate were removed through sorting. The sprout length of the germinated rice ranged from 2 to 7 mm.

Drying and grinding

Both germinated and non-germinated samples were dried at 50 °C using a cabinet dryer for 3 h. The dried samples were then ground in a blender for 2 min (Philips HR2115, Philips, Netherlands). The ground samples were sieved through a 60-mesh sieve, vacuum-packaged, and stored at −18 °C prior to analysis [14,33].

Fatty acid analysis

Fatty acid analysis employed the modified method [16]. A 5 g of powdered sample were mixed with 7 mL chloroform, 14 mL methanol, and 5.6 mL distilled water. The mixture was then centrifuged at 4000 rpm for 10 min. Subsequently, 7 mL chloroform and 7 mL of 0.85% potassium chloride were added, followed by vortex mixing (Reax Top, Heidolph Instruments GmbH & Co. KG, Germany) and centrifugation (Sorvall Biofuge Primo, Thermo Fisher Scientific, USA) to achieve phase separation. The supernatant was filtered, then the chloroform phase was collected and evaporated under nitrogen. The extracted lipids were derivatized using BF_3–methanol to produce Fatty Acid Methyl Esters (FAMEs). The resulting FAMEs were analyzed using gas chromatography (GC) equipped with a flame ionization detector (FID). Separation was performed using a STABILWAX-DA 9000936 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The injector and detector temperatures were set at 220 °C and 240 °C, respectively. The initial oven temperature was set at 170 °C and held for 2 min, then increased to 220 °C at a rate of 8 °C/min and maintained for 5 min. The temperature was subsequently raised to 240 °C at 8 °C/min and held for 6 min. High-purity helium was used as the carrier gas at a flow rate of 33.6 mL/min. The external standard (FAME Mix C8–C24) was diluted with hexane to an appropriate concentration and then injected into the GC system under the same analytical conditions as those used for the samples. Fatty acid composition was identified by comparing the retention times of sample peaks with those of external standards.

$$\text{FA (mg/100 g sample)} = \frac{C \times V \times DF}{W_L} \times \frac{W_T}{W_S} \times 100$$

C = concentration of the quantified fatty acid obtained from the calibration curve (mg/L)
V = final volume of the FAME solution (L)
DF = dilution factor
W_L = total lipid extracted from the original sample (g)
W_T = mass of lipid used for FAME preparation (g)
W_S = initial sample weight (g)

Amino acid analysis

The analysis of total amino acids was performed utilizing the chromatographic methods [34,35]. A 60 mg sample was mixed with 4 mL of 6 N hydrochloric acid and hydrolyzed at 110 °C for 24 h. After cooling to room temperature (27 °C), the hydrolysate was neutralized with 6 N sodium hydroxide. Once the pH reached 7, distilled water was added to a final volume of 10 mL. The solution was then filtered using Whatman filter paper (0.2 μm). A 50 μL aliquot of the extract was derivatized with 300 μL of OPA (o-phthaldialdehyde) for 5 min. Subsequently, 20 μL of the derivatized sample was injected into the HPLC system. The separation process was conducted employing a gradient elution

system, with mobile phase A comprising methanol:50 mM sodium acetate:tetrahydrofuran (THF) (2:96:2, v/v/v) at a pH of 6.8, while mobile phase B was composed of methanol:water (65:35, v/v). The analytical procedure was executed using an HPLC system (Thermo Dionex UltiMate 3000, Thermo Fisher Scientific, USA) outfitted with a Hypersil GOLD™ column (4.6×250 mm, 5 μm). The flow rate was calibrated to 1.5 mL/min, with excitation and emission wavelengths set at 300 nm and 500 nm, respectively. The concentrations of amino acids were quantified by contrasting the chromatographic peak areas of the samples with those obtained from standard solutions.

GABA (γ-aminobutyric acid) analysis

GABA quantification was conducted utilizing a chromatographic method [36,37]. A 3 g sample was mixed with 5 mL of 80% ethanol and vortexed for 5 min (Reax Top, Heidolph Instruments GmbH & Co. KG, Germany). The mixture was then centrifuged at 5,000 rpm for 10 min at 4 °C (Sorvall Biofuge Primo, Thermo Fisher Scientific, USA). The supernatant was collected, and an additional 5 mL of 80% ethanol was added. The extraction was repeated, and the obtained supernatants were combined. The extract was subsequently evaporated using a vacuum rotary evaporator (RV10 Basic, IKA Works GmbH & Co. KG, Germany) for 30 min at a pressure of 65 mbar with gradual pressure reduction, a rotation speed of 90 rpm, and a temperature of 40 °C. The concentrated extract was reconstituted to 5 mL with distilled water and filtered through a 0.45 μm syringe filter. Prior to injection into the HPLC system, the samples were derivatized using o-phthaldialdehyde (OPA) reagent. A 20 μL aliquot of the derivatized solution was injected into the HPLC system. Gradient elution was implemented utilizing mobile phase A, which comprised methanol, 50 mM sodium acetate, and tetrahydrofuran (THF) in a ratio of 2:96:2 (v/v/v), alongside mobile phase B, consisting of methanol and water in a ratio of 65:35 (v/v). The analytical process was conducted using an HPLC system (Shimadzu LC-10AD Liquid Chromatography, Japan) that was equipped with a fluorescence detector (Shimadzu RF-10A XL, Japan) and a LiChrosphere 100 RP-18 column (4.6×150 mm, 5 μm). The flow rate was maintained at 1.5 mL/min, with excitation and emission wavelengths established at 300 and 500 nm, respectively. The concentration of GABA was ascertained by comparing the chromatographic peak areas of the samples with those of the corresponding standards.

γ-Oryzanol analysis

γ-Oryzanol analysis was performed according to a chromatographic method with minor modifications [38]. A 0.5 g sample was dissolved in 10 mL of methanol and sonicated at room temperature (27 °C) for 60 min using ultrasonic bath (BK-2000, BAKU, China). The extract was then filtered through a 0.45 μm syringe filter prior to injection into the HPLC system (Shimadzu Prominence, Japan) equipped with a diode array detector (SPD-M20A) and an Agilent Zorbax Eclipse XDB-C18 column (4.6×150 mm, 5 μm). Isocratic elution was performed utilizing a solvent system composed of acetonitrile and methanol in a volumetric ratio of 60:40. The volume of the sample injected was 20 μL, and the duration of the analytical run was established at 30 min. The concentration of γ-oryzanol was quantified at an optical wavelength of 325 nm through the comparative analysis of the chromatographic peak areas of the experimental samples in relation to those of the established standard. Under this analytical condition, γ-oryzanol was detected as individual steryl ferulates.

Vitamin E analysis

The concentration of vitamin E was measured by a colorimetric assay following the AOAC method [39]. The absorbance was measured at 520 nm using UV-Vis spectrophotometer (Thermo Scientific Genesys 30, USA). Total tocopherol-equivalent content was quantified by referring the sample absorbance to the standard calibration curve.

Vitamin K analysis

Vitamin K determination was conducted by chromatography according to a previously reported method, with slight modifications in the extraction procedures [40]. Approximately 4 g of sample was extracted with 14 mL of n-hexane in a brown centrifuge tube. Menaquinone-3 was added solely as an internal standard. The mixture was shaken for 5 min using shaker (SR-II, Taiyo Scientific Industrial, Japan) and then centrifuged for 5 min (4,000 rpm, 4 °C) using a centrifuge (CF16RXII, Hitachi, Japan). The hexane

layer was collected and evaporated under vacuum using a centrifugal vacuum concentrator (Taitec Corporation, Japan) for 60 min (1,800 rpm, 40 °C). Subsequently, 2 mL of n-hexane was added to the sample and vortexed, followed by purification using a Sep-Pak Silica Cartridge (Waters Corporation, USA). The cartridge was preconditioned with n-hexane, after which the sample was loaded and eluted with a mixture of diethyl ether:n-hexane (4:96, v/v). The eluted fraction was collected and evaporated under vacuum again for 30 min (1,800 rpm, 40 °C). Next, 200 µL of ethanol was added to the sample, vortexed for 1 min (Vortex-Genie 2, Scientific Industries, USA), and sonicated for 1 min (Sharp, Japan). After cooling on ice for 15 min, the sample was filtered through a 0.5 µm syringe filter (DISMIC-03JP, Advantec, Toyo Roshi Kaisha, Japan). A 50 µL aliquot was injected into an HPLC system (Agilent Technologies, USA) equipped with a fluorescence detector (Agilent 1260 Infinity, USA), using a Puresil C18 column (4.6×150 mm, 5 µm) and an RC-10 reduction column (Shiseido, Japan). The mobile phase consisted of methanol:ethanol (80:20, v/v). Vitamin K1 (phylloquinone) analysis was performed at a flow rate of 1.0 mL/min with excitation and emission wavelengths set at 240 and 430 nm, respectively. Phylloquinone content was determined by comparing the chromatographic peak areas obtained from the sample with those of the standard. The entire extraction and analysis procedures were conducted under minimal light conditions.

Statistical analysis

Data were processed using SPSS version 16.0 (SPSS Inc., USA). All analyses were performed in triplicate, and the results are reported as mean ± standard deviation. Statistical differences between samples were assessed using one-way analysis of variance (ANOVA) with a significance threshold of $p < 0.05$. Homogeneous data were further compared using Duncan's multiple range test (DMRT), whereas non-homogeneous data were analyzed using a paired Student's t-test.

Results and discussion

Fatty acid

Fatty acids in the samples were identified based on the comparison of their methyl ester derivatives with the C8–C24 Fatty Acid Methyl Esters (FAME) standard mixture. Of the 17 fatty acids targeted in this analysis, eight compounds were detected and quantified in the rice extracts, expressed as mg/100 g sample (**Table 1**). Both IR-64 brown rice and Cempo Ireng black rice showed comparable fatty acid profiles in terms of the types of compounds present. In terms of quantity, differences were observed between non-germinated and germinated treatments in both varieties. A post-germination increase was observed in both IR-64 brown rice and Cempo Ireng black rice.

The increase in fatty acid content during germination is associated with enhanced lipolytic enzyme activity, which hydrolyzes lipid into fatty acids and glycerol [41]. **Figure 1** indicates that germination increased the total fatty acid content by 1.3-fold in IR-64 brown rice from 2,133.88 to 2,703.39 mg/100 g and by 1.7-fold in Cempo Ireng black rice from 1,454.26 to 2,458.39 mg/100 g, suggesting a greater responsiveness of Cempo Ireng black rice to germination in terms of fatty acid accumulation. The changes in fatty acid content observed in this study are consistent with previous findings on germinated rice. Kim *et al*. [42] reported that unsaturated fatty acids levels in brown rice increased after germination. Similarly, Albarracín *et al*. [13] found higher polyunsaturated fatty acid (PUFA) levels in germinated Fortuna-type rice. In this study, oleate, linoleate, and palmitate were the major fatty acids detected, which is comparable to earlier findings on the fatty acid composition of different rice varieties from Sri Lanka and Japan [43,44].

Table 1 Fatty acid profile of rice (mg/100 g).

Fatty acid	NG-IR	G-IR	NG-CI	G-CI
miristate	12.37 ± 2.20^{b}	16.89 ± 0.82^{a}	3.82 ± 0.2^{d}	7.23 ± 0.10^{c}
palmitate	501.48 ± 188.84^{a}	463.75 ± 33.46^{a}	228.72 ± 33.46^{b}	363.02 ± 21.42^{ab}
stearate	62.27 ± 29.17^{a}	52.05 ± 4.49^{ab}	28.28 ± 4.49^{b}	41.91 ± 1.99^{ab}

Fatty acid	NG-IR	G-IR	NG-CI	G-CI
arachidate	11.57 ± 1.18^{ab}	14.67 ± 1.10^{a}	8.90 ± 0.84^{b}	12.18 ± 4.29^{ab}
palmitoleate	3.09 ± 0.63^{c}	5.72 ± 0.29^{a}	2.21 ± 0.67^{c}	4.38 ± 0.11^{b}
oleate	954.09 ± 102.84^{b}	1,305.87 ± 49.63^{a}	661.55 ± 25.74^{c}	1,026.22 ± 58.06^{b}
linoleate	555.75 ± 58.14^{c}	796.14 ± 25.74^{b}	488.79 ± 31.56^{c}	937.79 ± 54.17^{a}
linolenate	33.26 ± 3.09^{c}	48.30 ± 2.18^{b}	31.98 ± 3.41^{c}	65.68 ± 3.32^{a}
ω-6/ω-3 ratio	16.70 ± 0.26^{c}	16.49 ± 0.24^{c}	15.33 ± 0.63^{b}	14.27 ± 0.10^{a}

Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

As shown in **Table 1**, the ratio of omega-6/omega-3 in IR-64 brown rice and Cempo Ireng black rice ranged from 14.27 to 16.70, which is below the threshold value of 20 and therefore considered unlikely to contribute to the development of non-insulin-dependent diabetes mellitus [45]. In Cempo Ireng black rice, germination resulted in a favorable reduction in the ratio of omega-6/omega-3. Compared to other plant-based foods such as spelt [46], oat [47], and buckwheat [48], the ratio of omega-6/omega-3 in IR-64 brown rice and Cempo Ireng black rice was more favorable.

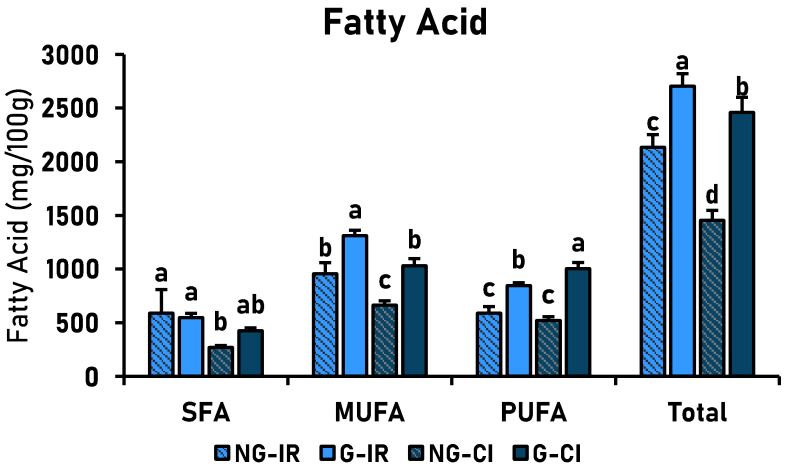


Figure 1 Fatty acid profile of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng. SFA, MUFA, and PUFA represent saturated, monounsaturated, and polyunsaturated fatty acids, respectively.

As shown in **Figure 1**, germination resulted in a notable increase in both monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in IR-64 brown rice and Cempo Ireng black rice. In IR-64 brown rice, MUFA and PUFA levels increased by 37.03% and 43.37%, respectively, from 957.18 to 1,311.60 mg/100 g and from 589.01 to 844.44 mg/100 g. Similarly, in Cempo Ireng black rice, MUFA and PUFA contents increased by 52.82% and 92.69%, respectively, from 663.76 to 1,014.34 mg/100 g and from 520.77 to 1,003.47 mg/100 g. The fatty acid composition of both IR-64 brown rice and Cempo Ireng black rice, before and after germination, was predominantly composed of monounsaturated fatty acids (MUFAs). A greater abundance of MUFAs in comparison with saturated fatty acids (SFAs) may contribute to a lower susceptibility to chronic inflammation and obesity-associated metabolic complications, including type 2 diabetes (T2D), hepatic steatosis, and cardiovascular diseases [49].

Amino acid

The amino acid analysis revealed the presence of 14 amino acids in IR-64 brown rice and Cempo Ireng black rice across both treatment conditions, before and after germination. These consisted of seven essential amino acids, specifically histidine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine, and seven non-essential amino acids, including arginine, tyrosine, aspartic acid, glutamic acid, serine, glycine, and alanine, as shown in **Table 2**. The amino acid data reported in this study represent total amino acids. Germination caused no significant change in the overall amino acid composition of either rice variety in this study. Similar observations were reported by Albarracin *et al*. [13] and He *et al*. [17], who found that the amino acid profiles of Fortuna-type and Japonica rice remained relatively unchanged after germination. Although the expected increase in total amino acid after germination was not observed, the absence of a significant decrease indicating that this process does not compromise protein-related nutritional quality and may still be considered a viable approach.

Table 2 Amino acid profile of rice (mg/100 g).

Amino acid	NG-IR	G-IR	NG-CI	G-CI
histidine	307.49 ± 91.42^{a}	231.78 ± 3.47^{a}	246.84 ± 7.68^{a}	246.04 ± 1.20^{a}
methionine	147.01 ± 20.82^{a}	102.31 ± 31.98^{a}	118.74 ± 36.64^{a}	107.02 ± 19.22^{a}
valine	310.73 ± 20.72^{a}	323.54 ± 16.63^{a}	332.85 ± 34.67^{a}	348.48 ± 22.21^{a}
phenylalanine	459.66 ± 39.67^{a}	450.42 ± 24.98^{a}	445.89 ± 38.41^{a}	458.63 ± 18.37^{a}
isoleucine	234.62 ± 24.91^{a}	254.14 ± 6.22^{a}	248.12 ± 36.44^{a}	264.67 ± 14.63^{a}
leucine	689.47 ± 70.57^{a}	713.22 ± 9.96^{a}	679.39 ± 74.62^{a}	706.7 ± 21.03^{a}
lysine	392.15 ± 27.45^{a}	363.59 ± 7.19^{a}	400.32 ± 41.67^{a}	407.23 ± 27.14^{a}
arginine	349.75 ± 4.03^{a}	329.63 ± 14.92^{a}	341.37 ± 15.04^{a}	342.72 ± 12.19^{a}
tyrosine	351.94 ± 6.85^{ab}	336.32 ± 9.62^{a}	347.80 ± 8.50^{ab}	363.45 ± 13.25^{a}
aspartic acid	813.79 ± 19.09^{a}	789.92 ± 18.48^{a}	783.84 ± 10.74^{a}	788.59 ± 10.77^{a}
glutamic acid	1,539.47 ± 39.13^{a}	1,483.74 ± 37.26^{ab}	1,441.68 ± 42.31^{b}	1,419.94 ± 26.21^{b}
serine	527.66 ± 4.57^{a}	483.5 ± 31.21^{b}	504.99 ± 6.03^{ab}	498.86 ± 3.64^{ab}
glycine	539.68 ± 12.46^{a}	491.10 ± 15.95^{b}	511.27 ± 15.88^{b}	499.67 ± 5.78^{b}
alanine	545.04 ± 2.78^{a}	517.52 ± 11.44^{b}	528.5 ± 5.15^{b}	525.55 ± 3.73^{b}

Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

As presented in **Table 2**, the dominant amino acids found in both IR-64 brown rice and Cempo Ireng black rice were glutamic acid, aspartic acid, and leucine. In IR-64 brown rice, the measured total amino acid content was 7,208.47 ± 131.11 mg/100 g before germination and 6,870.73 ± 160.30 mg/100 g after germination. Meanwhile, Cempo Ireng black rice contained 6,931.60 ± 290.51 mg/100 g total amino acids in the non-germinated sample and 6,977.55 ± 182.65 mg/100 g in the germinated sample. This suggests that germination had no significant effect on total amino acid content. Gunathunga *et al.* [50] reported that germination mainly increases free amino acids rather than total amino acids. Compared with previous reports, the amino acid content observed in this study exceeded those found in Japonica rice [17], Fortuna-type rice [13], FARO 44 [14], and NERICA-8 [15] which were 7.73, 99.5, 65.76 - 77.15, and 64.25 - 72.72 mg/100 g, respectively. In addition, the total amino acid content detected in this study was within the typical range reported for rice varieties from Sri Lanka, which is 6,450 - 9,600 mg/100 g [51].

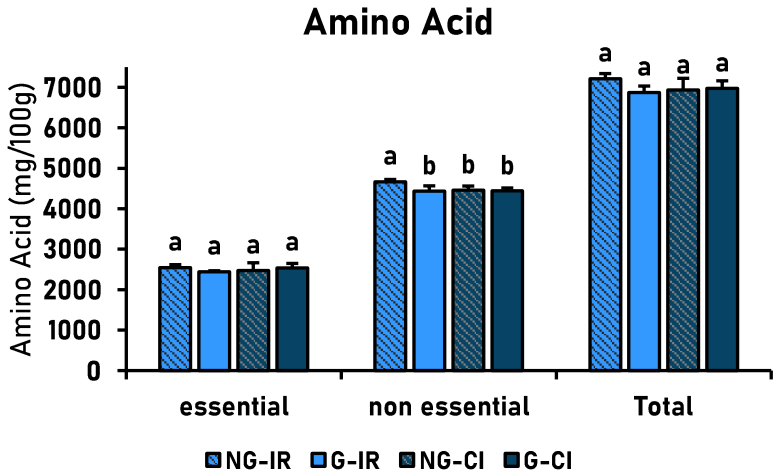


Figure 2 Amino acid profile of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

A slight decrease in non-essential amino acids was observed in IR-64 brown rice after germination, as can be seen in **Figure 2**. Serine, glycine, and alanine exhibited a reduction in IR-64 brown rice following germination (**Table 2**). These amino acids are closely associated with energy production through their conversion into pyruvate and subsequent entry into the TCA (tricarboxylic acid) cycle [52,53]. This suggests differences in metabolic allocation during germination between IR-64 brown rice and Cempo Ireng black rice. While primary metabolism is essential in both varieties, IR-64 brown rice may allocate more resources toward energy production, leading to a greater utilization of these amino acids [54]. In contrast, Cempo Ireng black rice, which is rich in secondary metabolites, may distribute its metabolic resources more broadly, such us for energy production, phenolic biosynthesis, and antioxidant activity, resulting in a more stable amino acid profile [55].

GABA (γ-aminobutyric acid)

As a non-proteinogenic amino acid, GABA has been associated with improved β-cell viability and may also participate in glucagon secretion regulation through its role as a paracrine factor [22]. Germination increased the GABA content by 2.8-fold in IR-64 brown rice and 5-fold in Cempo Ireng black rice, from 9.74 ± 1.23 mg/100 g to 27.19 ± 3.55 mg/100 g and from 13.46 ± 3.31 mg/100 g to 66.98 ± 7.54 mg/100 g, respectively, as shown in **Figure 3**. This increasing trend is consistent with previous studies reported in Hom Nil and Niew Dam rice [10], IPB 3S, Inpari 17, Inpari 42, Inpari 43, and Situ Bagendit rice [16], Japonica-type rice [17], FARO 44 rice [14], and NERICA-8 rice [15].

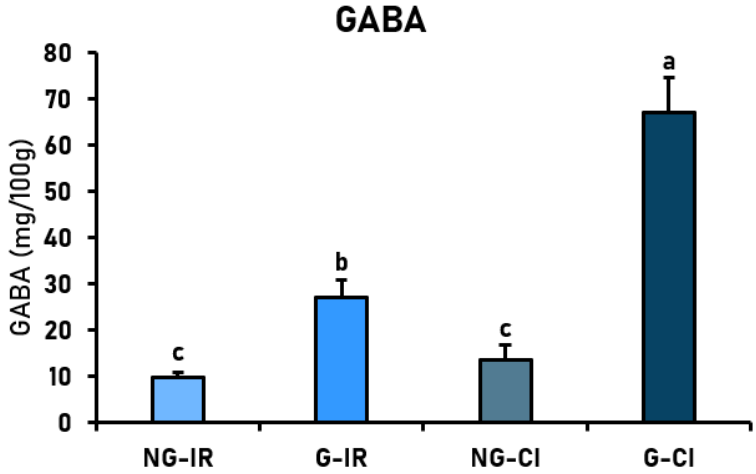


Figure 3 GABA content of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

The higher GABA content observed after germination is likely associated with increased glutamate decarboxylase (GAD) activity, which converts glutamate into GABA and promotes its accumulation [56,57]. The difference in the magnitude of GABA increase between the two rice varieties cannot be attributed to differences in glutamate availability. As shown in **Table 2**, the glutamic acid levels in IR-64 brown rice and Cempo Ireng black rice were comparable. Therefore, the variation in GABA accumulation may be associated with differences in the regulation or activity of GAD and the GABA shunt pathway among rice varieties during germination. GABA biosynthesis in germinated rice depends greatly on enzyme activity and germination-related physiological responses, both of which may be influenced by genetic variation among rice varieties [58].

Earlier studies documented GABA contents of 38 ± 21 mg/100 g, 179 ± 15 mg/100 g, 220.13 ± 8.57 mg/100 g, 7.62 ± 1.14 mg/100 g, and 6.95 ± 0.34 mg/100 g in Hom Nil, Niew Dam [10], Japonica-type [17], FARO 44 [14], and NERICA-8 [15], respectively. Compared with these data, the GABA contents measured in IR-64 and Cempo Ireng rice were higher than those of FARO 44 and NERICA-8 [23,43], but still lower than that found in Niew Dam [10] and Japonica-type rice [17]. When compared with other germinated plants, germinated Cempo Ireng black rice contained more GABA than waxy wheat [59], corn [60], purple corn [61], and white waxy corn [62], which were reported to contain 15.5, 27 - 28, 53.17, and 7 - 9.5 mg/100 g GABA, but still lower than that reported in germinated red bean, which was 443.57 mg/100 g [63].

γ-Oryzanol

γ-Oryzanol is a phytochemical naturally present in rice and consists of various steryl ferulates, including cycloartenyl ferulate, 2,4-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate [64]. It has been recognized for its pronounced antioxidant and anti-inflammatory effects [23]. In the present study, germination promoted γ-oryzanol accumulation in both rice varieties, as illustrated in **Figure 4**, with the values expressed as mg/100 g sample. Total γ-oryzanol in IR-64 brown rice increased from 384.52 ± 7.77 mg/100 g to 469.14 ± 18.42 mg/100 g, equivalent to a 22% rise. In Cempo Ireng black rice, the total γ-oryzanol increased by 25%, from 862.29 ± 53.98 mg/100 g to 1,085.00 ± 38.16 mg/100 g. Similar trends were observed for individual steryl ferulates, including campesteryl ferulate, β-sitosteryl ferulate, 2,4-methylenecycloartanyl ferulate, and cycloartenyl ferulate.

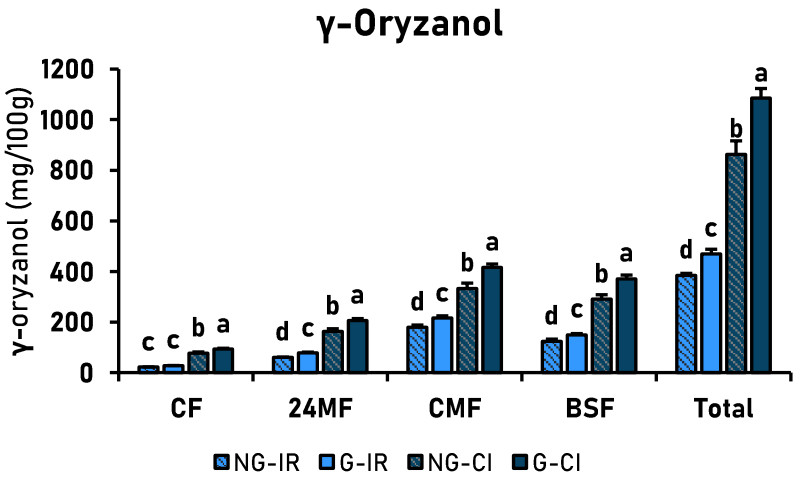


Figure 4 γ-oryzanol content of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng. CF, 24MF, CMF, and BSF represent cycloartenyl ferulate, 2,4-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate, respectively.

Campesteryl ferulate increased by 1.2-fold in IR-64 brown rice, from 179.93 ± 9.07 mg/100 g to 215.37 ± 9.02 mg/100 g, and by 1.3-fold in Cempo Ireng black rice, from 331.99 ± 21.74 mg/100 g to 415.58 ± 13.62 mg/100 g. β-sitosteryl ferulate increased by 1.2-fold in IR-64 brown rice, from 123.49 ± 8.78 mg/100 g to 149.42 ± 4.82 mg/100 g, and by 1.3-fold in Cempo Ireng black rice, from 290.42 ± 17.36 mg/100 g to 370.52 ± 15.05 mg/100 g. 2,4-methylenecycloartanyl ferulate increased by 1.3-fold in both varieties, from 60.41 ± 0.75 mg/100 g to 77.60 ± 3.32 mg/100 g in IR-64 brown rice and from 163.15 ± 10.13 mg/100 g to 205.69 ± 7.81 mg/100 g in Cempo Ireng black rice. Cycloartenyl ferulate increased by 1.2-fold in both varieties, from 21.70 ± 0.50 mg/100 g to 26.76 ± 1.29 mg/100 g in IR-64 brown rice and from 76.74 ± 4.79 mg/100 g to 93.21 ± 3.04 mg/100 g in Cempo Ireng black rice.

The mechanism underlying the increase in γ-oryzanol during germination has been rarely reported. The positive trend observed in this study may be associated with water absorption during germination, which activates biological processes and enhances the availability of bioactive compounds [65]. Furthermore, Hayashi and Satoh [66] reported that acetyl-CoA levels increase during germination. Given its role as a key precursor, the elevated availability of acetyl-CoA may contribute to enhanced γ-oryzanol biosynthesis in rice [52,65].

The increase in γ-oryzanol observed in this study aligns with previous reports on RD-8 rice [19] and Buebang 3 Morchor [18], in which germination enhanced γ-oryzanol accumulation. In contrast, Munarko *et al*. [16] observed a decrease or no significant alteration in γ-oryzanol levels during 48 h of germination across five rice varieties. These inconsistent responses may be associated with varietal differences, since germination effects are known to vary depending on rice genotype [52]. Notably, germinated Cempo Ireng black rice contained more γ-oryzanol than IR-64 brown rice and other rice varieties reported in the literature, such as RD-8 [19], IPB 3S, Inpari 42, Inpari 43, Inpari 17, Situ Bagendit, [16], Kum Jao Morchor 107 and Buebang 3 Morchor [18].

Vitamin E

Vitamin E belongs to the group of lipid-soluble compounds and has been widely associated with antioxidant and anti-inflammatory functions [24]. Vitamin E is commonly found in cereal grains, particularly during seed desiccation, storage, and germination, where it functions to protect membrane lipids from photooxidative damage [67]. As shown in **Figure 5**, germination decreased vitamin E (tocopherol) content by 1.4-fold in IR-64 brown rice, from 15.05 ± 0.21 mg/100 g to 10.74 ± 0.16 mg/100 g, and by 1.6-fold in Cempo Ireng black rice, from 38.99 ± 0.21 mg/100 g

to 24.96 ± 0.10 mg/100 g. This reduction may be attributed to the role of tocopherols as antioxidants in preventing oxidative damage during the early stages of germination [18]. A similar decreasing trend has been observed in NERICA-8 rice [15]. An increase in vitamin E content may require a longer germination time [68].

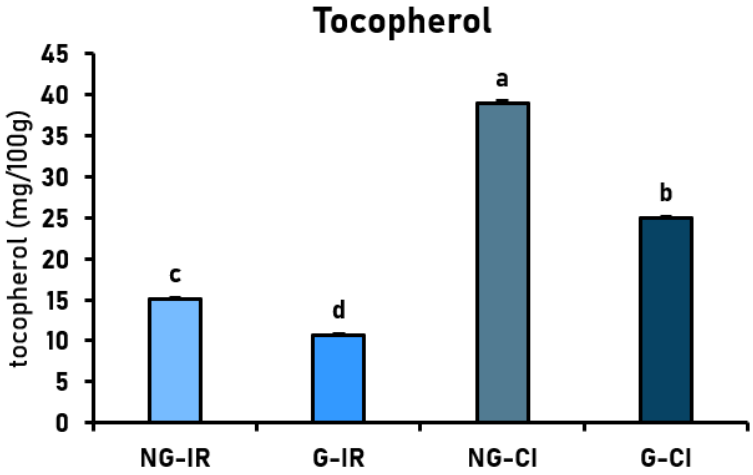


Figure 5 Total tocopherol-equivalent content of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

Despite the observed reduction, the vitamin E levels in IR-64 brown rice and Cempo Ireng black rice remain relatively higher compared to those reported for other rice varieties, including RD-8, FARO 44, NERICA-8, and Buebang 3 Morchor. Specifically, vitamin E contents reported for RD-8 [19], FARO 44 [14], and NERICA-8 [15] were 1.25 ± 0.02 mg/100 g, 2.00 ± 0.14 mg/100 g, and 1.89 ± 0.14 mg/100 g, respectively, while Buebang 3 Morchor contained 10.26 ± 0.21 mg/100 g [18]. However, IR-64 brown rice and Cempo Ireng black rice contained lower vitamin E levels than Kum Jao Morchor 107, which has been reported to contain 36.48 ± 2.04 mg/100 g [18].

Cempo Ireng black rice contained a higher level of vitamin E than IR-64 brown rice. This result is in accordance with earlier studies [18,69], which reported that pigmented rice varieties generally contain higher vitamin E levels than non-pigmented rice. In addition, the vitamin E levels measured in the present study exceeded those previously reported for several cereal and pseudocereal crops, including wheat [70], quinoa [71], and sweet corn [72], with reported levels of 2.25 - 3.37, 5.82, and 1.51 mg/100 g, respectively.

Vitamin K

Vitamin K is a lipid-soluble vitamin primarily recognized for its involvement in blood coagulation [25]. This vitamin is generally divided into two major forms: phylloquinone, referred to as vitamin K1, and menaquinone, referred to as vitamin K2 or MK [73]. Vitamin K1 is commonly abundant in green leafy vegetables, whereas vitamin K2 is mainly found in animal-based and fermented products [74]. Previous study has reported the presence of vitamin K1 in cereals, cereal bars, and oatmeal [75]. Unpolished rice may also contain vitamin K; however, research on this vitamin K in unpolished rice remains limited [5].

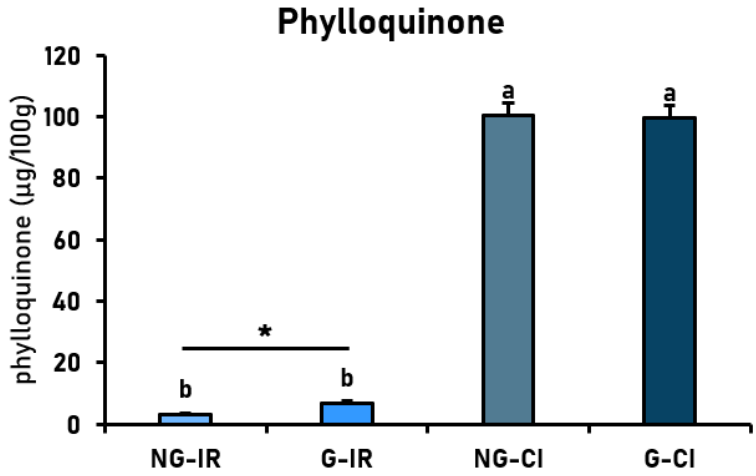


Figure 6 Phylloquinone content of rice. Values bearing different superscript letters are significantly different at $p < 0.05$, values marked with * are significantly different ($p < 0.05$) according to Student's t-test, n = 3. NG-IR: non-germinated IR-64; G-IR: germinated IR-64; NG-CI: non-germinated Cempo Ireng; G-CI: germinated Cempo Ireng.

Figure 6 shows that germination did not significantly affect the phylloquinone content in IR-64 brown rice and Cempo Ireng black rice. In IR-64 brown rice, the phylloquinone content was 3.18 ± 0.41 µg/100 g prior to germination and 6.81 ± 0.77 µg/100 g following germination, while in Cempo Ireng black rice, the values were 100.46 ± 3.82 µg/100 g and 99.68 ± 3.87 µg/100 g, respectively. Although this difference did not reach statistical significance ($p > 0.05$), IR-64 brown rice exhibited an approximately 2-fold increase after germination according to Student's t-test, suggesting a potential positive trend.

The phylloquinone levels measured in the present study exceeded those previously reported in crude rice bran which was reported to contain 1.9 µg/100g [6]. Nevertheless, it remained lower than the levels reported in rice bran oil which ranged from 26 to 568 µg/100g [76]. When compared with other food sources, IR-64 brown rice showed phylloquinone levels comparable to those of wheat breads [75], Kuruluthuda rice, and Pachchaperumal rice [77], with reported values of 6.7, 5.03, and 6.93 µg/100g, while Cempo Ireng black rice exhibited values close to those found in canola oil and cabbage which were reported to contain 127 and 145 µg/100 g phylloquinone, respectively [78].

Conclusions

Germination induced biochemical changes in both IR-64 brown rice and Cempo Ireng black rice. The changes observed between the two rice varieties were quite distinct, suggesting possible fundamental differences in metabolic allocation during the germination process. These differences may be associated with rice variety and/or differences in bran pigmentation (pigmented vs. unpigmented rice). Germination increased the total fatty acid content in both varieties, with Cempo Ireng black rice exhibiting a favorable reduction in the ratio of omega-6/omega-3. In contrast, germination did not affect the total amino acid content of either variety, although a slight decrease in non-essential amino acids was observed in IR-64 brown rice, indicating that this process does not compromise protein-related nutritional quality and can still be considered a viable approach. Furthermore, germination enhanced the levels of γ-aminobutyric acid (GABA) and γ-oryzanol in both rice varieties. Conversely, vitamin E (tocopherol) content decreased in both varieties, which may be related to its antioxidant function during the initial phase of germination. Regarding vitamin K1 (phylloquinone), no statistically significant differences were observed; however, a significant 2-fold increase was observed in IR-64 brown rice based on Student's t-test, suggesting a potential positive trend. Overall, these results provide meaningful evidence about the biochemical changes induced by germination in different rice varieties and highlight its potential for enhancing nutritional quality in the development of functional foods. These additional data may provide a

stronger scientific basis for the future development of functional food products derived from IR-64 brown rice and Cempo Ireng black rice, such as snack bars, rice crackers, instant drink, and complementary foods for infants.

Acknowledgements

This research received financial support from the Indonesia Endowment Fund for Education (LPDP) scholarship (SKPB2491/LPDP/LPDP.3/2024) by Ministry of Finance of the Republic of Indonesia. The authors gratefully acknowledge the Laboratory of Food and Nutrition, Universitas Gadjah Mada, Indonesia, and the Laboratory of Nutrition, Tohoku University, Japan, for their support in providing research facilities.

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools, specifically ChatGPT by OpenAI, in the preparation of this manuscript for language editing and grammar correction. No content was generated, and no data were interpreted using AI. The authors take full responsibility for the content and conclusions of this work.

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Erina Wulandari: Methodology; Formal Analysis; Investigation; Validation; Writing - Original Draft; Visualization; Funding Acquisition. **Lulum Leliana**: Validation; Writing - Review & Editing; Supervision. **Tanno Yuta**: Validation; Formal Analysis; Investigation. **Afifah Zahra Agista**: Validation; Supervision. **Hitoshi Shirakawa**: Resources; Writing - Review & Editing; Supervision. **Yusuke Ohsaki**: Validation; Formal Analysis; Supervision. **Wahyu Dwi Saputra**: Supervision; Project Administration; Conceptualization; Writing - Review & Editing.

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