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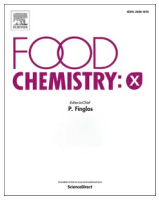

# Germination alters the bioactive compounds of pigmented and non-pigmented rice varieties in fresh and year-old stored seeds

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ABSTRACT

This study assessed the effect of germination on bioactive compounds in fresh and one-year-old seeds of pigmented and non-pigmented rice varieties. Rice varieties had similar changes in bioactive compounds during germination. GABA and several phenolic acids increased 11.7 to 18.5 folds and 0.7 to 4.2 folds, respectively in germinating seeds. The vitamin E compounds, γ-tocotrienol and α-tocopherol increased by 23 to 35 % only in soaked seeds, but declined after seed germination. In the germinating seeds, higher levels of phenolic acids (e.g., protocatechuic acid, 0.5-fold higher) and some compounds of vitamin E (e.g., γ-tocotrienol, 0.4-fold higher) were observed in the pigmented rice compared to the non-pigmented line, in accordance with antioxidant activity. Additionally, one-year-old seeds exhibited the same increases in bioactive compounds as the fresh seeds. We conclude that the seeds of the pigmented variety have the potential as a raw material for germination in the rice industry.

## 1. Introduction

Germination has been shown to improve the nutritional quality of cereal grains, including rice. The health benefits of germinated seeds have been well established (Chaiyasut et al., 2016; Wu et al., 2013). At present, germinated rice is not only consumed as a staple food in Thailand, Japan and India but is also used as a functional ingredient for in various foods (e.g., desserts and functional beverages) (Castanho et al., 2023; Jabeen et al., 2024; Michaitrakun et al., 2023). Hence, raw rice seed, being naturally enriched with bioactive compounds, has been gaining increasing attention.

Rice varieties with high amounts of bioactive compounds are now widely available on the market. Rice can be classified into two main categories, pigmented and non-pigmented, based on the presence of colored phenolic compounds in the pericarp layer. Kum Jao Morchor 107 and Baebang 3 Morchor, representatives of the pigmented and non-pigmented types, are two popular traditional rice varieties grown in Northern Thailand. Previous studies have shown that these two varieties contain high amounts of bioactive compounds (e.g., anthocyanins, phenolics, flavonoids and tocopherols), and thus have potential applications in food, pharmaceutical, and cosmetic products (Kittipongpatana et al., 2024; Mapoung et al., 2023; Ruksiriwanich et al., 2023; Thongkong et al., 2023). However, how the bioactive compounds in these varieties are influenced by germination has not been studied. Although these rice varieties are rich in bioactive profiles, germination can cause fluctuation in the levels of some compounds, and this can reflect on the potential market value. Increases in gamma-aminobutyric acid (GABA) and phenolic compounds have been demonstrated in germinating seeds, while some water-soluble phenolic compounds and fat-soluble compounds, such as vitamin E, can be decreased by soaking and germination (Kong et al., 2022; Wu et al., 2022). Therefore, the bioactive compounds present during germination should be compared between the non-pigmented and pigmented rice varieties when assessing their potential applications.

Rice seed characters (e.g., fresh vs. aged seeds) are important for potential germination. Aged rice seeds usually have low germination rates due to the deterioration of the seed structure and the loss of chemical components during storage (Shu et al., 2021). The loss of nutritional and antioxidant compounds such as proteins, essential oils, and phenolics has been reported in stored rice seeds (Naveed et al., 2023; Yamuangmorn et al., 2018). These reasons lead to the limited utilization of aged rice seeds in the food manufacturing industry.

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Nonetheless, it is unknown whether aged rice seeds would show differences in the contents of bioactive compounds from fresh seeds when germinated under the same conditions. The main hypothesis of this study is that the variety and history of rice seeds will affect the changes in bioactive compounds during germination.

The objective of this study was to investigate the effect of germination on the bioactive compound contents of two traditional rice varieties in fresh and one-year-old seeds. Germinated seeds prepared from pigmented and non-pigmented seeds were chosen based on specific active compounds targeted for future food application. Recognizing the advantages of fresh and year-old seeds will provide new information concerning seed utilization for the rice industry.

## 2. Materials and methods

### 2.1. Experimental design

A completely randomized 2 × 3 factorial design (two rice varieties × three rice samples) was arranged with three replications. Two traditional rice varieties were employed, Kum Jao Morchor 107 (KJ CMU107), a pigmented rice variety, and Buebang 3 Morchor (BB3 CMU), a non-pigmented rice variety. The three rice treatments were a control (unsoaked and non-germinated seeds), soaked (soaked with non-germinated seeds), and germinated (soaked with germinated seeds). Seeds of the pigmented and non-pigmented rice varieties were harvested in different cropping seasons, with fresh seed harvested in 2021 and one-year-old seed harvested in 2020. Seeds from both cropping seasons were produced at the same research field station (Chiang Mai University, Chiang Mai, Thailand) and at the same time of the year during the rainy season from July to November. The pre- and post-harvest management practices were similar during both cropping seasons. Rice seeds were stored in a cold room at 16 °C until use in January 2022.

### 2.2. Germination procedure

Fresh and year-old seeds in the form of paddy rice were dehusked to produce brown rice seeds prior to germination. The brown rice seeds were soaked in water at 30 °C for 12 h before being incubated at 35 °C with 95 % moisture content for 24 h. The germination rate was calculated by weighing the rice seeds with a radicle. The germination rates of fresh seed were 93.1 % and 95.8 % for pigmented and non-pigmented rice varieties, respectively. The germination rates of year-old seeds were 44.7 % and 2.8 % for pigmented and non-pigmented rice varieties, respectively. Brown rice samples were collected and treated as raw (no germination), soaked, and germinated, then dried in a freeze dryer for 24 h. Fig. 1 shows the brown rice samples of the two traditional rice varieties germinated in the form of fresh seeds. About 30 g of dried sample was mechanically ground in a vibrating cub mill (Fritsch, PULVERISETTE 9) and stored at −35 °C until analysis.

### 2.3. Analyses of gamma oryzanol and vitamin E contents

The rice extract was prepared based on a previously published method (Sirithunyalug et al., 2018). Briefly, rice grains (0.5 g) were subjected to a heating–cooling process at 65 °C for 20 min and 2–8 °C for 20 min to inactivate endogenous lipase as it may catalyze the esterification of gamma-oryzanol, and then extracted by absolute methanol solvent (99.8 % methanol) in a shaking incubator at 60 °C for 1 h. Then, the γ-oryzanol was determined by reverse-phase HPLC using an Agilent model 1200 apparatus (Santa Clara, CA, USA) under the same conditions described by Sirithunyalug et al. (2018). Briefly, a 250 mm × 4.6 mm diameter Symmetry Shield RP18 column was obtained from Waters Co., Ltd. (NSW, Australia). The mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid in a ratio of 50:44:3:3 with a flow rate of 1.0 mL/min at a 330 nm detection wavelength. All samples were tested in triplicate.

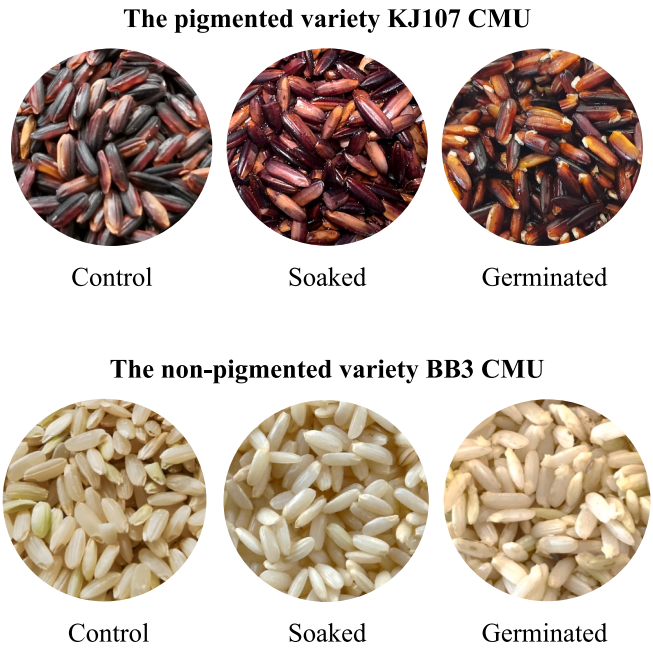


**Fig. 1.** The appearance of the rice samples from the control (unsoaked and non-germinated seeds), soaked (soaked with non-germinated seeds), and germinated (soaked with germinated seeds) seeds of pigmented (KJ CMU107) and non-pigmented (BB3 CMU) rice varieties from fresh seed samples.

Additionally, eight isoforms of vitamin E, including δ-, β-, γ-, α-tocotrienol and δ-, β-, γ-, α-tocopherol contents, were determined by reverse-phase HPLC by using an Agilent 1200 with a fluorescence detector as described by Pengkumsri et al. (2015). All compounds were detected at the excitation and emission wavelengths of −296 and 330 nm. A KINETEX®PFP column (diameter 4.6 mm × 150 mm) (Phenomenex Co., Ltd., Torrance, CA, USA) was employed, with the mobile phase consisting of methanol and de-ionized water in a ratio of 9:1 at a flow rate of 0.6 mL/min. All samples were analyzed in triplicate.

### 2.4. Analysis of γ-aminobutyric acid (GABA)

GABA was determined using the method of Le et al. (2020) with modification. The derivatization reagent was composed of methanolic ortho-phthalaldehyde (OPA), borate buffer (0.5 M, pH 9.9), and 2-mercaptoethanol (2-MCE) in the ratio of 40:10:1 by volume. The derivatization of free amino acids in the standard and tested samples was performed by mixing 114 μL of the derivatization reagent with 36 μL of standard or test samples of extract or standard. The derivatization reaction was carried out at 22 °C for one minute. The HPLC system was an Agilent 1200 series with a fluorescence detector (Agilent Technologies, Germany). The mobile phase consisted of 0.05 M sodium acetate (pH 7.2), 0.1 M sodium acetate, acetonitrile, and methanol in the ratio of 25:35:30:10 by volume, and the flow rate was set at 1 mL/min. The excitation and emission wavelengths of the fluorescence detector were 340 nm and 455 nm, respectively.

### 2.5. Analysis of anthocyanins

Anthocyanins were extracted and analyzed based on a previously published method (Sirilun et al., 2022). Briefly, rice grains (0.5 g) were extracted with 80 % ethanol, pH 2.0, and the extract was evaporated under reduced pressure and dried under a vacuum before analysis. Cyainidin-3-glucoside, peonidin-3-glucoside, cyanidin, and peonidin were determined by reverse-phase HPLC using an Agilent 1200 equipped with a multiwavelength detector and a Symmetry RP18 Column (4.6 mm × 250 mm) (Waters Co., Ltd., Milford, USA). The gradient

elution mobile phases were 3 % phosphoric acid in acetonitrile and deionized water at the flow rate of 1.0 mL/min. The detection wavelength was set at 520 nm. All samples were tested in triplicate.

### 2.6. Analysis of phenolic and flavonoid compounds

Reverse-phase HPLC was used to analyze phenolic and flavonoid compounds as previously described by Phromnoi et al. (2021). The mobile phase consisted of 30 % acetonitrile in 0.1 % acetic acid and deionized water at the flow rate of 1.0 mL/min, and a Symmetry Shield RP18 column (4.6 mm × 250 mm, 5 μm particle diameter, Waters Co., Ltd.) was used for separation of each compound. The UV detector was set at 278 and 325 nm, and the amounts of each detected compound in the samples were calculated and expressed as mg/g extract.

### 2.7. Analysis of antioxidant activity

Antioxidant capacity was determined by the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The DPPH and FRAP assays were performed following the methods of Amarowicz et al. (2004) and Benzie and Strain (1996), respectively, with some modifications as described in Yamuangmorn et al. (2018). Rice flour (0.1 g) was extracted with 10 mL of the absolute methanol solvent. The extract was placed on an orbital shaker (IKA KS 250 B) for 30 min and then separated by centrifugation (MSE Super Minor, England) at 3396 ×*g* for 10 min and filtered through a 0.22 μmNylon syringe filter. The reaction mixture contained 0.3 mL sample extract, 1.6 mL methanol and 0.5 mL of 0.1 mmol $L^{-1}$ DPPH solution. Blank tubes were prepared using 0.3 mL supernatant and 2.1 mL of methanol. The mixtures were shaken, incubated in the dark at room temperature for 20 min, and the absorbance was measured with a spectrophotometer at 517 nm. For FRAP analysis, briefly, 2.0 mL of extracted solution was transferred into a 25 mL volumetric flask, and 20 mL of 0.1 M sodium acetate (pH 4.0), 0.5 mL of 0.5 % (*w/v*) phenanthroline, and 0.5 mL of 0.3 mM Fe (III) were added. Blanks of the extracts were performed using samples as above, except 0.5 % (w/v) phenanthroline was not added and samples were diluted with 0.1 M sodium acetate (pH 4.0). After incubating in a 37 °C water bath for 20 min, the absorbance at 510 nm was measured.

### 2.8. Statistical analysis

Differences between treatments were analyzed by ANOVA followed by LSD tests. All statistical analyses were carried out using Statistix (version 10). A $p$-value <0.05 was considered significant.

**Table 1**
GABA, γ-oryzanol, tocotrienols, and tocopherol (mg/100 g) in fresh and year-old seeds according to the germination process (G) and variety (V).

| | Fresh seed | | | *p*-Value | | | Year-old seed | | | *p*-Value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pigmented rice | Non-pigmented rice | mean | G | V | G × V | Pigmented rice | Non-pigmented rice | mean | G | V | G × V |
| GABA | | | | 0.0000 | 0.0427 | 0.0107 | | | | 0.0000 | 0.0014 | 0.0009 |
| control | 1.10 ± 0.04$^{D}$ | 1.59 ± 0.05$^{D}$ | 1.35C | | | | 1.19 ± 0.03$^{D}$ | 1.45 ± 0.03$^{D}$ | 1.32C | | | |
| soaked | 5.47 ± 0.03$^{C}$ | 5.11 ± 0.24$^{C}$ | 5.29B | | | | 5.26 ± 0.01$^{C}$ | 4.93 ± 0.20$^{C}$ | 5.09B | | | |
| germinated | 21.47 ± 0.24 $^{A}$ | 20.18 ± 0.47$^{B}$ | 20.83 A | | | | 20.83 ± 0.32 $^{A}$ | 19.07 ± 0.07$^{B}$ | 19.95 A | | | |
| mean | 9.35 A | 8.96b | | | | | 9.09a | 8.48b | | | | |
| γ-oryzanol | | | | 0.0000 | 0.0000 | 0.0000 | | | | 0.0000 | 0.0000 | 0.0000 |
| control | 1003.34 ± 7.69$^{B}$ | 320.41 ± 6.56$^{F}$ | 661.88 B | | | | 1008.0 ± 38.31$^{C}$ | 347.89 ± 10.97$^{E}$ | 677.95 A | | | |
| soaked | 839.79 ± 7.08$^{C}$ | 407.63 ± 11.68$^{E}$ | 623.70C | | | | 860.74 ± 19.92$^{B}$ | 484.92 ± 12.64$^{D}$ | 672.83 A | | | |
| germinated | 760.31 ± 7.54$^{D}$ | 1022.66 ± 13.91 $^{A}$ | 891.49 A | | | | 753.34 ± 26.50 $^{A}$ | 332.05 ± 12.03$^{E}$ | 542.70B | | | |
| mean | 867.81 A | 583.57b | | | | | 874.29 A | 388.29b | | | | |
| δ-tocotrienol | | | | 0.0000 | 0.0000 | 0.0000 | | | | 0.0000 | 0.0000 | 0.0000 |
| control | 4.68 ± 0.07$^{C}$ | 3.89 ± 0.02$^{D}$ | 4.29B | | | | 3.00 ± 0.16$^{E}$ | 3.82 ± 0.07$^{D}$ | 3.41B | | | |
| soaked | 2.76 ± 0.01$^{E}$ | 5.00 ± 1.13$^{B}$ | 3.88C | | | | 5.10 ± 0.03 $^{A}$ | 4.28 ± 0.08$^{B}$ | 4.69 A | | | |
| germinated | 4.68 ± 0.42$^{C}$ | 6.02 ± 0.32 $^{A}$ | 5.35 A | | | | 2.98 ± 0.04$^{E}$ | 3.99 ± 0.04$^{C}$ | 3.48B | | | |
| mean | 4.02b | 4.97a | | | | | 3.70b | 4.03a | | | | |
| γ-tocotrienol | | | | 0.0000 | 0.0000 | 0.0000 | | | | 0.0000 | 0.0000 | 0.0000 |
| control | 140.12 ± 0.74$^{C}$ | 81.19 ± 0.61$^{F}$ | 110.66C | | | | 110.23 ± 2.16$^{B}$ | 78.55 ± 0.65$^{E}$ | 94.39C | | | |
| soaked | 154.59 ± 0.22 $^{A}$ | 114.42 ± 1.13$^{D}$ | 134.51 A | | | | 148.79 ± 0.50 $^{A}$ | 85.06 ± 0.46$^{C}$ | 116.93 A | | | |
| germinated | 143.95 ± 1.19$^{B}$ | 105.66 ± 0.32$^{E}$ | 124.81B | | | | 111.61 ± 0.29$^{B}$ | 81.52 ± 0.75$^{D}$ | 96.57B | | | |
| mean | 146.22a | 100.42b | | | | | 123.54a | 81.71b | | | | |
| α-tocotrienol | | | | 0.0000 | 0.0008 | 0.0000 | | | | 0.0000 | 0.0058 | 0.0000 |
| control | 7.34 ± 0.34$^{C}$ | 15.19 ± 1.00$^{B}$ | 11.27 A | | | | 4.98 ± 0.63$^{C}$ | 13.34 ± 1.18$^{B}$ | 9.16 A | | | |
| soaked | 0.00 ± 0.00$^{D}$ | 19.36 ± 0.65 $^{A}$ | 9.68B | | | | 0.00 ± 0.00$^{D}$ | 16.34 ± 0.58 $^{A}$ | 8.17B | | | |
| germinated | 7.64 ± 0.16$^{C}$ | 15.10 ± 1.03$^{B}$ | 11.37 A | | | | 4.83 ± 0.25$^{C}$ | 15.08 ± 1.02 $^{A}$ | 9.96 A | | | |
| mean | 7.49b | 16.55 A | | | | | 4.90b | 14.92 A | | | | |
| α-tocopherol | | | | 0.0000 | 0.0000 | 0.0000 | | | | 0.0000 | 0.0000 | 0.0000 |
| control | 42.87 ± 0.22$^{B}$ | 7.17 ± 0.20$^{E}$ | 25.02B | | | | 28.26 ± 1.66$^{B}$ | 10.98 ± 0.32$^{E}$ | 19.62B | | | |
| soaked | 50.90 ± 0.83 $^{A}$ | 11.07 ± 0.80$^{D}$ | 30.99 A | | | | 43.59 ± 0.29 $^{A}$ | 13.69 ± 0.28$^{D}$ | 28.64 A | | | |
| germinated | 36.48 ± 2.04$^{C}$ | 10.26 ± 0.21$^{D}$ | 23.37C | | | | 20.69 ± 0.18$^{C}$ | 9.23 ± 0.15$^{F}$ | 14.96C | | | |
| mean | 43.42a | 9.50b | | | | | 30.85 A | 11.30b | | | | |

Mean values ± SD (n = 3). Uppercase letters indicate significant differences among the germination treatments (raw, soaked, and germinated), and lowercase letters indicate significant differences between varieties (KJ CMU107 and BB3 CMU) ($p < 0.05$). Superscript letters indicate significant differences among germination processes and varieties ($p < 0.05$).

## 3. Results

### 3.1. GABA

The GABA contents of pigmented and non-pigmented rice varieties in the fresh and year-old seeds are listed in Table 1. In the fresh seed, there was a significant increase in the GABA contents of the two rice varieties during germination. In the soaked samples, the GABA content was increased 4.0 and 2.2-fold in pigmented and non-pigmented rice varieties, respectively, compared to the control sample without soaking or germination. There was a sharp increase in the germinated samples; the contents were increased 18.5 and 11.7-fold in the pigmented and non-pigmented rice, respectively. Similarly, the year-old seeds showed a significant increase in GABA content in both varieties. The content of GABA in the soaked samples was increased by factors of 4.4 and 3.4 in the pigmented and non-pigmented rice, respectively compared to the control, and by factors of 17.5 and 13.2, respectively, in the germinated seeds.

### 3.2. γ-Oryzanol

The effects of germination on the γ-oryzanol content of pigmented and non-pigmented rice in the fresh and year-old seeds are presented in Table 1. Germination significantly affected the γ-oryzanol content in fresh seed differently between the two varieties. In pigmented rice, there was a reduction of γ-oryzanol in the soaked and germinated samples by 16 % and 24 %, respectively, compared to the control. In contrast to non-pigmented rice, there was a 21 % increase in the γ-oryzanol content in the soaked seeds and a 219 % increase in the germinated seeds. Furthermore, the content of GABA in the year-old seeds was significantly affected by the germination process and rice variety (Table 1). In pigmented rice, the γ-oryzanol content was reduced in both soaked and germinated seeds, by 15 % and 25 %, respectively, compared to the control. In contrast, the content of γ-oryzanol in non-pigmented rice was increased by 39 % in the soaked seeds.

### 3.3. Vitamin E

Vitamin E compounds comprising of tocotrienols and tocopherols s in the form of δ-, γ-, α- and β- of the pigmented and non-pigmented rice were analyzed, and the results are presented in Table 1. There were three δ-, γ-, and α-tocotrienols, and one form of α-tocotrienol detected in this study. In the fresh seed, the contents of δ-, γ-, and α-tocotrienols were significantly affected by the germination process and rice variety. For example, the content of γ-tocotrienol in both soaked and germinated samples was increased by 10 % and 3 %, respectively in the pigmented rice compared to the control, whereas greater increases of 45 % and 30 % were observed in the non-pigmented rice. In contrast, the change in the δ-tocotrienol content differed between treatments and varieties. The δ-tocotrienol content in the pigmented rice decreased by 41 % in the soaked seeds but increased in the germinated sample to the initial content before germination. However, in the non-pigmented rice, increases of 29 % and 55 % were observed in the soaked and germinated samples, respectively. The α-tocopherol content in the pigmented rice increased by 19 % in the soaked seeds, but declined in the germinated seeds, reaching a level lower than the control. However, α-tocopherol content in the non-pigmented rice was increased by 54 % in the soaked seeds but remained unchanged in the germinated seeds.

In the year-old seeds, the contents of δ-, γ-, and α-tocotrienols were also significantly affected by germination and rice variety, and the changes of these compounds had similar trends as the fresh seed. For example, the content of γ-tocotrienol increased by 35 % and 8 % in the soaked seeds of the pigmented and non-pigmented rice varieties but declined in the germinated seeds. However, the final content in the germinated seeds was higher than in the control. For α-tocopherol, the contents in pigmented and non-pigmented rice decreased by 27 % and 16 % in germinated seeds, respectively, even though the content was increased in the soaked seeds. In the germinated groups of both fresh and year-old seeds, the contents of γ-tocotrienol and α-tocopherol were higher in the pigmented rice than the non-pigmented rice, whereas the contents of δ- and α-tocotrienols were higher in the non-pigmented rice.

### 3.4. Phenolic compounds

The results for phenolic compounds, including protocatechuic acid, caffeic acid, ferulic acid, and syringic acid, are presented in Table 2. For the fresh seed, germination and rice variety significantly affected the protocatechuic acid, caffeic acid, and ferulic acid contents, without a significant interaction between the two factors. Increases of 0.4, 3.5, and 4.2-fold were observed in the germinated seed for protocatechuic acid, caffeic acid, and ferulic acid, respectively, compared to the control, while no significant difference of these compounds was observed between soaked and control groups. The pigmented rice showed higher contents of protocatechuic acid and caffeic acid than the non-pigmented rice, but the non-pigmented rice had a higher content of ferulic acid. There was a small decrease in syringic acid in germinated seed.

Similar increases in phenolic acids were observed in the year-old seed during germination. The contents of protocatechuic acid, caffeic acid, and ferulic acid were significantly affected by the germination process and rice variety, with respective increases of 0.8, 4.1, and 3.7-fold compared to the control in germinated seed, while the contents in the soaked seed remained unchanged. Additionally, the pigmented rice had higher contents of protocatechuic acid and caffeic acid compared to the non-pigmented rice, while the content of ferulic acid was higher in the non-pigmented rice. In contrast, the content of syringic acid was slightly decreased in germinated seed compared to the control.

### 3.5. Flavonoid compounds

The effect of germination on the contents of rutin, quercetin, kaempferol, apigenin, and luteolin of pigmented and non-pigmented rice varieties in fresh and year-old seeds are presented in Table 3. In the fresh seed, the contents of rutin and quercetin were significantly affected by the germination process and rice variety. An average decrease of 18 % in the rutin content was observed in the germinated seeds compared to the control. In contrast, the quercetin content increased by approximately 38 % in the germinated seeds. Comparisons between the two rice varieties showed that the non-pigmented rice had higher rutin and quercetin contents than the pigmented rice. Moreover, the content of luteolin was slightly increased in the germinated seed, although the content was not detectable in the control and soaked seeds. The changes in kaempferol and apigenin contents were not statistically significant between germination treatments and rice variety.

The changes in flavonoid compounds of two rice varieties in the year-old seeds during germination were similar to those observed in the fresh seeds. There was an approximate decrease of 12 % in the rutin content in the germinated seeds. In contrast, the quercetin content of the germinated seed increased by 42 % compared to the control. Overall, the non-pigmented rice had higher contents of rutin and quercetin than the pigmented rice. Luteolin was observed only in the germinated seed, not in the control or soaked treatments. The contents of kaempferol and apigenin were not significantly different during germination.

### 3.6. Anthocyanin compounds

The contents of cyanidin-3-glucoside and peonidin-3-glucoside in the pigmented rice in fresh and year-old seeds are presented in Table 4. In the fresh seeds, the contents of cyanidin-3-glucoside and peonidin-3-glucoside were significantly reduced during germination. The content of cyanidin-3-glucoside was reduced by 21 % in the soaked seeds compared to the control and by 61 % in the germinated seeds. Similarly, there was a reduction in the peonidin-3-glucoside content in the soaked

**Table 2**
Phenolic compounds (μg/1 g) in fresh and year-old seeds according to germination process (G) and variety (V).

| | Fresh seed | | | *p*-Value | | | Year-old seed | | | *p*-Value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pigmented rice | Non-pigmented rice | mean | G | V | G × V | Pigmented rice | Non-pigmented rice | mean | G | V | G × V |
| Protocatechuic acid | | | | 0.0000 | 0.0000 | 0.1836 | 45.23 ± 1.75 | 28.72 ± 1.42 | 36.98B | 0.0000 | 0.0000 | 0.3317 |
| control | 46.45 ± 19.0 | 31.99 ± 3.11 | 39.22B | | | | 43.44 ± 2.51 | 27.22 ± 2.29 | 35.28B | | | |
| soaked | 46.93 ± 2.00 | 28.65 ± 1.52 | 37.79B | | | | 75.03 ± 2.51 | 55.45 ± 1.92 | 65.24 A | | | |
| germinated | 78.19 ± 2.45 | 58.33 ± 1.84 | 68.26 A | | | | 54.57a | 37.13b | | | | |
| mean | 57.19 A | 39.66b | | | | | | | | | | |
| Caffeic acid | | | | 0.0000 | 0.0075 | 0.0637 | | | | 0.0000 | 0.0001 | 0.0559 |
| control | 23.94 ± 1.49 | 22.87 ± 1.37 | 23.40B | | | | 21.25 ± 1.74 | 18.87 ± 1.66 | 20.06B | | | |
| soaked | 21.51 ± 1.21 | 19.86 ± 2.20 | 20.69C | | | | 19.80 ± 1.95 | 15.65 ± 0.83 | 17.72B | | | |
| germinated | 110.2 ± 2.17 | 103.41 ± 2.76 | 106.81 A | | | | 107.46 ± 2.52 | 95.64 ± 2.45 | 101.55 A | | | |
| mean | 51.88a | 48.71b | | | | | 49.50a | 43.39b | | | | |
| Syringic acid | | | | 0.0000 | 0.0053 | 0.1740 | | | | 0.0000 | 0.4486 | 0.2162 |
| control | 19.12 ± 0.39 | 19.26 ± 0.62 | 19.19 A | | | | 18.31 ± 0.41 | 17.89 ± 0.35 | 18.10 A | | | |
| soaked | 18.34 ± 0.43 | 19.36 ± 0.46 | 18.85 A | | | | 17.75 ± 0.29 | 17.28 ± 0.54 | 17.52 A | | | |
| germinated | 16.68 ± 0.34 | 17.78 ± 0.58 | 17.28B | | | | 15.74 ± 0.39 | 16.13 ± 0.72 | 15.93B | | | |
| mean | 18.05b | 18.80a | | | | | 17.27 | 17.10 | | | | |
| Ferulic acid | | | | 0.0000 | 0.0009 | 0.0635 | | | | | | |
| control | 3.25 ± 0.28 | 3.80 ± 0.28 | 3.53B | | | | 2.84 ± 0.22 | 3.50 ± 0.33 | 3.17B | 0.0000 | 0.0001 | 0.0611 |
| soaked | 2.83 ± 0.27 | 3.38 ± 0.27 | 3.11B | | | | 2.33 ± 0.12 | 3.10 ± 0.17 | 2.72B | | | |
| germinated | 16.34 ± 1.30 | 20.58 ± 1.30 | 18.46 A | | | | 12.62 ± 0.77 | 16.89 ± 1.18 | 14.75 A | | | |
| mean | 4.47b | 9.25a | | | | | 5.93b | 7.83a | | | | |

Mean values ± SD (n = 3). Uppercase letters indicate significant differences among the germination treatments (control, soaked, and germinated), and lowercase letters indicate significant differences between varieties (KJ CMU107 and BB3 CMU) ($p < 0.05$).

**Table 3**
Flavonoid compounds (μg/1 g) of fresh and year-old seeds according to germination process (G) and variety (V).

| | Fresh seed | | | *p*-Value | | | Year-old seed | | | *p*-Value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pigmented rice | Non-pigmented rice | mean | G | V | G × V | Pigmented rice | Non-pigmented rice | mean | G | V | G × V |
| Rutin | | | | | | | | | | | | |
| control | 10.26 ± 0.31 | 13.27 ± 0.44 | 11.77 A | 0.0000 | 0.0000 | 0.1378 | 9.49 ± 0.33 | 11.48 ± 0.45 | 10.47 A | 0.0000 | 0.0000 | 0.2147 |
| soaked | 10.85 ± 0.44 | 12.77 ± 0.46 | 11.81 A | | | | 9.62 ± 0.36 | 11.49 ± 0.44 | 10.55 A | | | |
| germinated | 8.29 ± 0.32 | 10.96 ± 0.45 | 9.63B | | | | 7.98 ± 0.52 | 10.42 ± 0.35 | 9.20B | | | |
| mean | 9.80b | 12.33a | | | | | 9.03b | 11.12a | | | | |
| Quercetin | | | | 0.0000 | 0.0000 | 0.0316 | | | | 0.0000 | 0.0001 | 0.0078 |
| control | 19.03 ± 0.82$^{E}$ | 21.05 ± 0.32$^{D}$ | 20.04B | | | | 18.09 ± 0.41$^{D}$ | 19.68 ± 0.30$^{C}$ | 18.89B | | | |
| soaked | 19.26 ± 0.45$^{E}$ | 22.42 ± 0.35$^{C}$ | 20.80B | | | | 18.18 ± 0.32$^{D}$ | 21.13 ± 0.29$^{B}$ | 19.66B | | | |
| germinated | 26.22 ± 0.62$^{B}$ | 27.41 ± 0.34 $^{A}$ | 26.82 A | | | | 25.77 ± 0.91 $^{A}$ | 26.06 ± 0.92 $^{A}$ | 25.92 A | | | |
| mean | 21.50a | 23.63b | | | | | 20.68b | 22.29a | | | | |
| Kaempferol | | | | 0.0624 | 0.0732 | 0.5942 | | | | | | |
| control | 14.70 ± 0.34 | 14.52 ± 0.43 | 14.61 | | | | 14.25 ± 0.42 | 13.23 ± 0.41 | 13.74 | 0.0621 | 0.6000 | 0.3068 |
| soaked | 14.95 ± 0.47 | 14.28 ± 0.40 | 14.61 | | | | 14.97 ± 0.34 | 13.33 ± 0.40 | 14.15 | | | |
| germinated | 15.37 ± 0.45 | 15.04 ± 0.50 | 15.21 | | | | 15.23 ± 0.48 | 13.66 ± 0.36 | 14.44 | | | |
| mean | 15.01 | 14.61 | | | | | 14.82 | 13.41 | | | | |
| Apigenin | | | | 0.0882 | 0.0700 | 0.3071 | | | | 0.0624 | 0.0610 | 0.1863 |
| control | 10.59 ± 0.38 | 11.25 ± 0.48 | 10.92 | | | | 10.25 ± 0.44 | 11.44 ± 0.42 | 10.85 | | | |
| soaked | 10.85 ± 0.32 | 10.99 ± 0.59 | 10.92 | | | | 10.53 ± 0.29 | 11.41 ± 0.55 | 10.97 | | | |
| germinated | 11.23 ± 0.69 | 12.21 ± 0.61 | 11.72 | | | | 11.04 ± 0.49 | 12.93 ± 0.44 | 11.99 | | | |
| mean | 10.89 | 11.48 | | | | | 10.61 | 11.93 | | | | |
| Luteolin | | | | | | | | | | | | |
| control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00B | 0.0000 | 0.4183 | 0.5137 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00B | 0.0000 | 0.2921 | 0.3312 |
| soaked | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00B | | | | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00B | | | |
| germinated | 1.16 ± 0.05 | 1.13 ± 0.07 | 1.15 A | | | | 1.02 ± 0.07 | 0.96 ± 0.06 | 0.99 A | | | |
| mean | 0.39 | 0.38 | | | | | 0.34 | 0.32 | | | | |

Mean values ± SD (n = 3). Uppercase letters indicate significant differences among the germination treatments (raw, soaked, and germinated), and lowercase letters indicate significant differences between varieties (KJ CMU107 and BB3 CMU) ($p < 0.05$). Superscript letters indicate significant differences among germination processes and varieties ($p < 0.05$).

and germinated seeds, with respective decreases of 26 % and 57 %.

For the year-old seeds, germination significantly decreased the contents of cyanidin-3-glucoside and peonidin-3-glucoside. Soaked and germinated seeds showed 22 % and 61 % reductions, respectively, in cyanidin-3-glucoside, and 27 % and 54 % for peonidin-3-glucoside compared to the control.

**Table 4**
Anthocyanins of pigmented rice variety (μg/1 g) in fresh and year-old seeds according to the germination process.

| | Fresh seed | *p*-Value | Year-old seed | *p*-Value |
|---|---|---|---|---|
| Cyanidin-3-glucoside | | 0.0000 | | 0.0000 |
| control | 69.29 ± 3.13a | | 56.71 ± 2.00a | |
| soaked | 54.47 ± 1.09b | | 44.14 ± 1.32b | |
| germinated | 27.05 ± 0.80c | | 22.18 ± 1.52c | |
| mean | 50.27 | | 41.01 | |
| Peonidin-3-glucoside | | 0.0000 | | 0.0000 |
| control | 5.85 ± 0.38b | | 4.84 ± 0.22a | |
| soaked | 4.29 ± 0.44b | | 3.53 ± 0.17b | |
| germinated | 2.50 ± 0.22a | | 2.18 ± 0.32c | |
| mean | 4.21 | | 3.52 | |

Mean values ± SD (n = 3). Lowercase letters indicate significant differences among germination processes (raw, soaked, and germinated) ($p < 0.05$).

### 3.7. Antioxidant activities

Antioxidant activities were determined by DPPH and FRAP assays in this study. In the fresh seeds, the germination process significantly affected the DPPH value differently between the two varieties (Fig. 2a). In the pigmented rice, the DPPH value declined by 28 % in the soaked seeds compared to the control; however, the value remained unchanged in the germinated seeds. In the non-pigmented rice, there was a slight non-significant change in the DPPH value. In contrast, there was a strong decrease in DPPH in the year-old seeds (Fig. 2b). The DPPH value in the pigmented rice was reduced by almost half in the soaked seeds, and then subsequently reduced by 68 % in the germinated seeds. No DPPH was detected in the non-pigmented rice. In the comparison between the two rice varieties, pigmented rice exhibited a higher DPPH value than non-pigmented rice.

The FRAP value in the fresh seeds was significantly affected by germination and variety (Fig. 2c). The values of pigmented rice decreased by 29 % and 57 % in the soaked and germinated rice seeds, respectively, compared to the control. The change in the FRAP values in the year-old seeds differed from those of the fresh seeds (Fig. 2d). Both soaked and germinated rice seeds had reduced FRAP values by approximately 13 % compared to the control, but no FRAP value was observed in a non-pigmented rice seed.

## 4. Discussion

Germination increased the contents of GABA and some phenolic acids. Several investigations have reported that germination significantly increased the contents of several bioactive compounds (Kong et al., 2022; Maksup et al., 2021; Michaitrakun et al., 2023). The alterations in bioactive compounds during seed germination result from a complex interplay of enzymatic activities, metabolic pathways, and regulatory gene expression (Maksup et al., 2021; Wunthunyarat et al., 2020). GABA, for instance, is primarily synthesized through the decarboxylation of glutamate, and its levels can fluctuate under different germination conditions (Yu et al., 2023). Furthermore, metabolic pathways like the phenylpropanoid pathway are upregulated, leading to the production of secondary metabolites. In this study, a significant increase in phenolic acids was observed, whereas flavonoid levels showed only minor changes throughout germination. These results contrast with the findings of Wu et al. (2022), who reported that the increments in phenolic acids and flavonoids were similar during germination. Although both phenolic acids and flavonoids are products of the phenylpropanoid pathway, they originate from distinct branches within this pathway (Corso et al., 2020). This difference may explain the distinct changes observed in phenolic acid and flavonoid compounds. The results suggest that flavonoid synthesis is either less responsive to the germination process or that its production is regulated at a slower rate, potentially requiring a longer germination period to observe significant changes.

The germination process led to a loss of vitamin E compounds in this study. Initially, vitamin E compounds, including δ-tocotrienol and α-tocopherol, increased in the soaked seeds, but it declined in germinated seeds. This may be explained by the fact that during seed soaking, rice seeds absorb water, which activates key enzymes, especially *MPBQ/MT2*, *γ-TMT*, and *TC*, in the vitamin E biosynthesis pathway (Kong et al.,

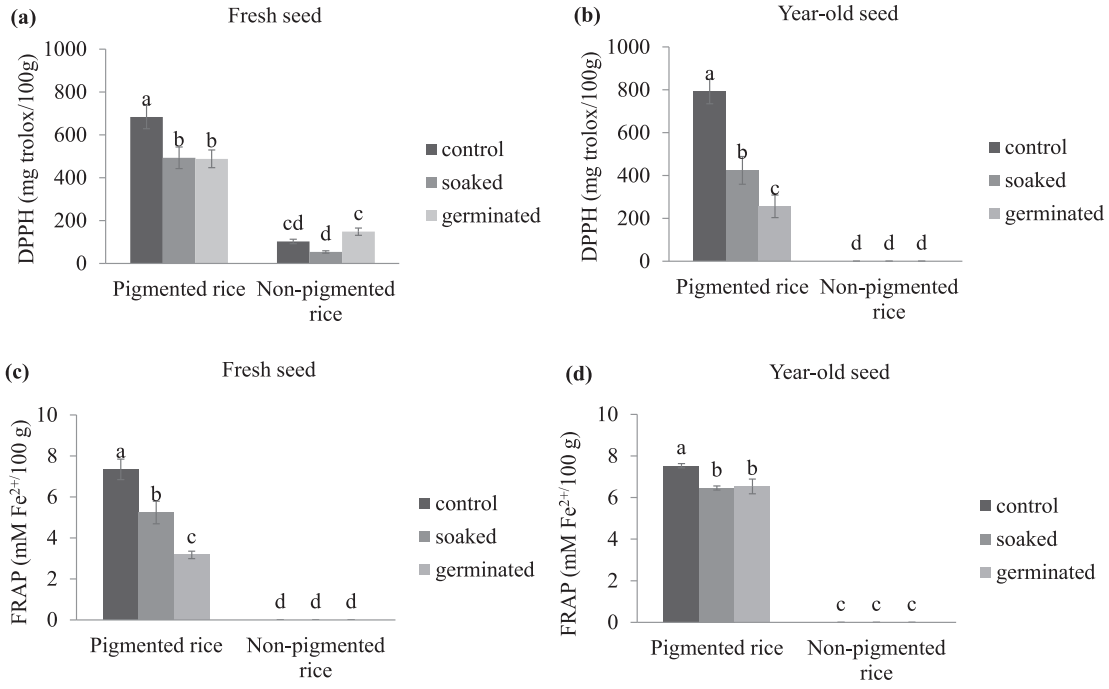


**Fig. 2.** Antioxidant activities (DPPH and FRAP) in fresh and year-old seeds according to germination process and variety. Mean values ± SD ($n = 3$). Different letters above the bars indicate significant differences among germination processes (control, soaked, and germinated) and varieties (pigmented and non-pigmented rice varieties) ($p < 0.05$).

2022). As germination progresses, the breakdown of stored compounds, such as lipids and proteins, provides energy for the growing seedling. During this process, some vitamin E compounds may be utilized to protect cellular membranes from lipid peroxidation. (Sattler et al., 2004). Some studies have shown that germination leads to an increase in vitamin E compounds (Kong et al., 2022), while others indicate a decrease in content (Pinheiro et al., 2021; Tarasevičienė et al., 2019). Based on these results, it can be inferred that soaking rice seeds followed by germination does not enhance vitamin E compounds, likely because vitamin E plays an antioxidant role in preventing oxidative damage during the early stages of seedling growth.

The present results demonstrate that both pigmented and non-pigmented rice varieties exhibited similar changes in the overall levels of bioactive compounds, such as GABA, phenolic acids, and flavonoids, after soaking and germination. Genetic variation in the contents of such compounds has been observed among rice varieties (Karladee & Suriyong, 2012). In this study, GABA increased during germination, and the pattern of increase was similar between the pigmented and non-pigmented varieties. The pigmented seed coat is associated with seed germination through water uptake and seed dormancy (Bhatt et al., 2016; Vidak et al., 2022; Zhang et al., 2023). From the results, the pigmented anthocyanins accumulated in the bran layer were unrelated to the GABA content, possibly because GABA is primarily synthesized in the embryonic tissue (Oh et al., 2019). This result was in accordance with a previous study reporting no marked increase in the GABA content between the pigmented and non-pigmented rice after germination (Karladee & Suriyong, 2012).

Additionally, the germination process did not result in a significant difference in the levels of phenolic acids and flavonoids between pigmented and non-pigmented rice varieties. The hypothesis that higher gene expression in the phenolic biosynthesis pathways of pigmented rice leads to greater contents of phenolic acids and flavonoids during seed germination remains inconclusive based on the findings of this study. Further research should quantify phenolic compounds and other secondary metabolites to explore possible synthesis mechanisms related to the nature of bioactive compounds in each type of seed (e.g., black, red, non-pigmented). Regarding changes in antioxidant activity, the DPPH values showed distinct patterns between pigmented and non-pigmented rice varieties. In pigmented rice, DPPH values decreased following soaking and germination, primarily due to the reduction in anthocyanin content. In contrast, DPPH values either remained stable or increased in non-pigmented rice. This finding suggests that the biochemical and metabolic changes occurring during soaking and germination differ between the two rice types, particularly in relation to the presence of anthocyanins. To gain a more comprehensive understanding of antioxidant activity in both pigmented and non-pigmented rice varieties, alternative methods such as 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) may be needed to accurately measure antioxidant capacity.

Interestingly, the results also demonstrated that fresh and year-old seeds exhibited different germination rates (3 % to 47 % for year-old seeds and above 90 % for fresh seeds) yet showed the same overall pattern of change in bioactive compounds. In contrast, the decrease in the seed germination rate was negatively correlated with the activity of enzymes involved in GABA synthesis and resulted in the restriction of GABA production during germination (Vipattanaporn et al., 2022). The modern rice variety with rapid germination produced GABA faster than the traditional rice variety with slow germination, but the final contents were equal after the proper germination time (Karladee & Suriyong, 2012). This study found that unlike the germination rate, the synthesis of GABA was not difference between fresh and year old seed during germination, suggesting that the year-old seed that exhibited a low germination rate could produce GABA and phenolic compounds at the maximum level under the appropriate germination conditions. The synthesis of GABA and phenolic compounds may have occurred prior to the phase of radicle protrusion, with increases in GABA and phenolic compounds observed at 24 h and 18 h after germination, respectively (Kamjijam et al., 2020; Wu et al., 2022). This study suggests that the germination rate may be not a key parameter for predicting the contents of bioactive compounds, while other germination traits, e.g., germination vigor or timing, should be considered for understanding the effect of the seed age on germination ability and the levels of bioactive compounds.

The fresh and aged seeds displayed differences in physical and chemical properties. Aged or stored seeds generally have damage to physical structures such as cell walls, embryos, and endosperm, factors that will impact the germination efficiency of rice seeds (Han et al., 2023; Wang et al., 2019). In addition, the degradation of saccharides and proteins caused by the lower respiration rate in the aged seed may result in lower nutrient values (Wang et al., 2019). The above discussion suggests that the loss in physical and chemical properties of fresh and aged seeds has an impact on germination efficiency, while this phenomenon does not affect the bioactive compounds of germinated seeds. A comparison of the results obtained for bioactive compounds between fresh and year-old seeds suggests that one-year-old seed had the highest performance for the improvement of nutritional values. However, in the usage of rice seeds by the food industry, one year should not be the storage limit to ensure the nutritional benefits of bioactive compounds and other nutrients in rice, but the longer storage may need to further evaluate for the stability.

## 5. Conclusions

Changes in most of the bioactive compounds in soaked and germinated seeds were similar between pigmented and non-pigmented rice varieties. Germination partly increased the contents of GABA and phenolic acids (protocatechuic acid, caffeic acid, and ferulic acid) in the germinated seeds, while flavonoid contents were slightly changed or remained unchanged during germination. γ-Oryzanol was contingent upon the variety and seed age, being increased only in the non-pigmented rice of fresh seed. Vitamin E compounds, including δ-tocotrienol and α-tocopherol, increased in the soaked seed, but the increase declined in the germinated seed. Reductions in anthocyanins occurred in both soaked and germinated seeds. Additionally, germination altered the levels of bioactive compounds according to the same pattern in fresh and year-old seeds. The findings contribute to the utilization of year-old seeds in that the storage can enhance the contents of bioactive compounds after germination. Considering the increasing levels of GABA and phenolic acids in the germinated seeds, this study has determined appropriate methods for the utilization of rice seeds according to variety and seed age for both health and economic benefits.

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## CRediT authorship contribution statement

**Supapohn Yamuangmorn:** Writing – original draft, Methodology, Investigation, Conceptualization. **Chalermpong Saenjum:** Validation, Resources, Investigation, Formal analysis. **Chanakan Prom-u-thai:** Writing – review & editing, Visualization, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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