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Exploring antioxidant and bioactive compounds of brown rice, germinated brown rice, and germinated Hang rice varieties

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Abstract

Rice is a staple food crop globally, playing a crucial role in feeding a large portion of the world's population. In addition to its nutritional value, rice contains bioactive compounds with significant biological effects. This study analyzed Gamma-aminobutyric acid (GABA), total phenolic compounds, proteins, and antioxidant properties in 24 rice extract samples, including anthocyanins in various pigmented rice varieties. GABA content ranged from 0.021 to 0.181 mg/g dry weight, with the highest levels in Kam-Noi germinated brown rice. The Folin-Ciocalteu method revealed total phenolic content ranging from 0.008 to 0.074 mg/g, with Mali-Daeng germinated brown rice showing the highest levels. Protein content, measured via Kjeldahl and SDS-PAGE methods, ranged from 6.090 to 8.380%, with Hom-Thung germinated brown rice having the highest protein concentration. Protein molecule sizes varied between 14.40 and over 97.00 kDa. Kam-Noi brown rice had the highest anthocyanin concentration. Antioxidant activity, evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous ion chelating (FIC), and oxygen radical absorbance capacity (ORAC) assays, showed that Nieow-Daeng germinated Hang rice had the strongest DPPH radical scavenging activity, while Hom-Thung germinated Hang rice chelated Fe^{2+} effectively, and Hom-Thung brown rice demonstrated the strongest peroxyl radical inhibition. These findings emphasize the presence of bioactive compounds and valuable antioxidant properties in rice.

1. Introduction

Rice, the queen of cereal crops, is highly valued for its abundant nutritional value, containing carbohydrates, protein, fat, dietary fiber, vitamins, as well as minerals, energy, fatty acids, and bioactive compounds. Rice is a basic food consumed by over 60% of the global population. Because rice contains, in addition to its nutritious components, a variety of phytochemicals that have been identified as bioactive substances with significant biological activity, such as antioxidant (Goufo and Trindade, 2014), anti-inflammatory (Samyor *et al.*, 2017), antihypertensive (Shobako and Ohinata, 2020) and anti-cancer characteristics (Kannan *et al.*, 2010). However, the amount of such substances and their biological activity depends on the species and where it is grown. Additionally, it also depends on pigment-containing and pigment-free variations and the types of rice, such as white rice, germinated Hang rice, germinated brown rice, and brown rice.

White rice is paddy rice from which the husks have been removed, and the rice has been polished to remove the membrane. Brown rice is rice that has not undergone the polishing process, that has had its husks removed. The rice germ and rice seed coat are still present. As a result, the rice has grains that are lighter in color and the same color as the rice husks. Germinated brown rice refers to

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brown rice that has undergone soaking in water. After that, work it until the roots start to emerge. Gamma-aminobutyric acid (GABA) is produced during the germination process when the enzyme glutamate decarboxylase breaks down glutamate (Hong and Kim, 2019). Hang germinated rice is produced through a process involving the germination of rice grains, followed by steaming until fully cooked, and then cracking and removing the husks (Phattayakorn *et al.*, 2016; Jandaruang and Preecharram, 2020). Furthermore, each variety of rice contains various biologically active substances, including phenolic compounds, carotenoids (such as α–, β–carotene, lutein, and lycopene), phytosterols, flavonoids (anthocyanin and proanthocyanidin), phytic acid, γ-oryzanol, coumaric acid, vitamins and minerals, along with other beneficial substances (Hudson *et al.*, 2000; Goufo and Trindade, 2014; Samyor *et al.*, 2017; Verma and Srivastav, 2020). Moreover, rice proteins are widely recognized for their hypoallergenic properties (Amagliani *et al.*, 2017; Pantoa *et al.*, 2020). Consequently, it is commonly utilized in the formulation of food for infants and individuals with gluten allergies. The diverse compounds found in rice serve medicinal purposes by helping to safeguard the body against harm. Therefore, consuming rice can be regarded as a form of medicinal nourishment, offering a safe and natural remedy.

Sakon Nakhon province boasts abundant natural resources, like as lush forests and mountains. This province is one of the five that make up the watershed areas of northeastern Thailand. The Phu Phan Mountain range encircles the plateau that makes up Sakon Nakhon province's overall terrain, which consists of plains in between valleys. A low, arable plain is in Sakon Nakhon province's middle region. Particularly in Mueang District, where a sizable Nong-Han pond serves as a vital supply of water for the province. Thus, the rice cultivated in Sakon Nakhon province is of high grade. The quantification of total phenolic compounds, GABA, anthocyanin, protein, and antioxidant activity in local rice varieties from Sakon Nakhon remains largely unexplored. This study investigates these compounds and assesses the biological activities in brown rice, germinated brown rice, and germinated Hang rice.

2. Materials and methods

2.1 Materials

There were eight types of rice (*Oryza sativa* L.) available: Chao-Daeng, Mali-Daeng, Mali-Bao, Kam-Noi, Som-Ma-Lee, Hom-Thung, Nieow-Daeng, and Pong-Aew (Figure 1(A–H)). All these samples were taken from Sakon Nakhon province, Kusuman district, Um-Chan subdistrict, and Ban Khok Sa-At, with taxonomy identification provided by the Sakon Nakhon Rice Research Center. The Folin-Ciocalteu phenol reagent, Gallic acid, Gamma-aminobutyric acid, Bovine serum albumin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, fluorescein and 2,2′-azobis-(2-methyl-propionamidine) dihydrochloride (AAPH) were acquired from Sigma-Aldrich Chemical Co., USA. In this experiment, substances of analytical grade were also utilized.

Figure 1. Characteristics of various rice varieties; (A) Kam-Noi, (B) Nieow-Daeng, (C) Mali-Bao, (D) Som-Ma-Lee, (E) Chao-Daeng, (F) Pong-Aew, (G) Mali-Daeng, and (H) Hom-Thung.

2.2 Sample preparation and extraction

The eight types of rice mentioned above were prepared as brown rice, germinated brown rice, and germinated Hang rice. The production procedure was as follows.

Brown rice was produced by milling and removing the husks from paddy, which still retain the rice bran and germ, without undergoing polishing. Preparation of germinated brown rice; the rice underwent a soaking process in water for 12 h at room temperature (28±5°C). After discarding the soaking water and rinsing, it was spread evenly over damp cheesecloth and then allowed to germinate through incubation. Following this, drying was completed using a hot air dryer set at 55°C for 10 h. The resulting dried rice (14% moisture) was then dehusked to produce germinated brown rice (Jandaruang and Preecharram, 2020). The germinated Hang rice was prepared by soaking paddy in water at room temperature (28±5°C) for 12 h, followed by draining. They were then incubated for 18 h, steamed with boiling water, and subsequently sun-dried. The dried grains were dehulled to produce germinated Hang rice (Jandaruang and Preecharram, 2020).

The 24 rice samples were extracted using 80% ethanol following the method described by Jandaruang and Preecharram (2020). Briefly, the sample was mixed with solvent at a ratio of 1:3 (w/v) and incubated with

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shaking at 150 rpm for 24 h. After incubation, the mixture was filtered to separate the solution, which was then concentrated by solvent evaporation. All 24 crude extract samples were stored at 4°C to analyze GABA, total phenolic compounds, anthocyanins, and antioxidant activity.

2.3 Gamma-aminobutyric acid content

The GABA contents were determined using the Jandaruang and Preecharram (2020) technique. The standard GABA content curve (y = 16.152X + 0.0122, R^2 = 0.9985) was used to compare the optical density reading to determine the GABA content in milligrams of GABA equivalents per gram dry weight.

2.4 Total phenolic compound content

A quantitative analysis of total phenolic components was conducted following the methodology outlined by Preecharram *et al*. (2024). The standard gallic acid content curve (y = 52.582X + 0.0387; R^2 = 0.9951) was utilized for comparing optical density readings to quantify the total phenolic content in milligrams of gallic acid equivalents per gram dry weight.

2.5 Anthocyanin content

The content of anthocyanin pigment in the rice pigment samples was quantified using the pH differential methods adapted from Moongngarm and Saetung (2010) and Konwatchara and Ahromrit (2014). Briefly, 50 µL of the sample extract was combined with 150 µL of pH 1.0 potassium chloride buffer, thoroughly mixed, and left to stand for 15 mins. Subsequently, the absorbance was measured at 515 and 700 nm using a UV-visible spectrophotometer with distilled water as a reference. Similarly, the extract was mixed with a sodium acetate buffer at pH 4.5, and absorbance was measured at the same wavelengths after 15 min.

Total anthocyanin content (mg/L) = (A × MW × DF × 1000) / e × l

where A = [(A515–A700) at pH 1.0 – (A515–A700) at pH 4.5], MW is molecular weight of cyanidin-3 glucoside (449.2 g/mol), DF is the dilution factor of sample, e is the molar absorptivity of cyanidin-3-glucosideequal to 26,900 and l is path length of cuvette in centimeters (cm).

2.6 Protein content

In crude protein analysis, the Kjeldahl method was employed to ascertain the nitrogen quantity, which was subsequently converted into protein content using the method described by Preecharram *et al*. (2024). The following formula can be used to determine the protein content;

Protein content (%) = N (%) × 6.25

N (%) = [1.4 × (Vs-Vb) × Conc. HCl] / weight of the sample (g)

where: Vs is the HCl volume for the titrating sample and Vb is the HCl volume for the titrating blank.

2.7 Molecular weight of protein

The rice protein fractions were extracted following the procedure outlined by Watanabe *et al.* (2019).

Protein extraction from rice and determination of its molecular mass using the SDS-PAGE method were conducted following the procedure outlined by Jandaruang and Preecharram (2020). Prior to protein molecular mass analysis via SDS-PAGE, protein concentration was assessed using the Bradford method, with Bovine Serum Albumin employed as the protein standard. The resulting calibration curve was represented by the equation y = 0.0181x + 0.0012, with an R^2 value of 0.9961.

2.8 Antioxidant activity

The antioxidant activity was assessed using the DPPH assay, the ferrous ion chelating (FIC) method and oxygen radical absorbance capacity (ORAC) assay.

The DPPH method involves direct binding to the free radical DPPH, whereas the FIC method involves binding to the metal ions that trigger the formation of free radicals. The ORAC assay quantifies the radical chain-breaking capacity of antioxidants by tracking the inhibition of peroxyl radical-induced oxidation. Peroxyl radicals, prevalent in lipid oxidation within both food and biological systems under physiological conditions, are key targets. Consequently, ORAC values are regarded by some as biologically meaningful metrics, serving as benchmarks for assessing antioxidant efficacy (Zhong and Shahidi, 2015).

The DPPH method was performed according to the procedure outlined by Preecharram *et al*. (2023).

The FIC method was conducted in accordance with the procedure delineated by Nurliyana *et al.* (2010). Briefly, the FIC method was as follows: the reaction mixture consisted of 250 µL samples at varying concentrations, 25 µL of 2 mM $FeCl_2$, and 800 µL of 70% ethanol. After thorough mixing, the mixtures were allowed to incubate for 5 min. Following this, 100 µL of 5 mM ferrozine was added, mixed thoroughly, and then incubated in the dark at room temperature for an additional 5 min. The absorbance of the Fe^{2+}-ferrozine complex was subsequently measured at 562 nm against

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blank (70% ethanol). The chelating ability was calculated using the formula:

Chelating ability (%) = [(Acontrol – Asample) × 100] / Acontrol,

where Acontrol is the absorbance of the reaction mixture containing only $FeCl_2$, 70% ethanol, and ferrozine, and Asample is the absorbance of the reaction mixture with crude extract, $FeCl_2$, 70% ethanol, and ferrozine. The experimental results are presented as the IC_{50} values.

ORAC analysis was conducted following the method outlined by Ou *et al.* (2001;2013). In brief, 150 µL of 1 mM fluorescein solution was combined with 25 µL of the sample solution and then incubated for 15 min at 37°C. Next, 25 µL of 153 mM AAPH was added, followed by shaking for 5 min. The resulting solution was subjected to fluorescence measurement using a fluorescent probe. Fluorescence readings were subsequently recorded every minute for a period of 30 min, employing an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The antioxidant capacity value (ORAC value) was calculated from the area under the curve (AUC). Both the area under the curve and the net area under the curve can be determined using the following equations:

The AUC was determined by computing the integral of the curve as $1 + f1/f0 + f2/f0 + f3/f0 + f4/f0 + \ldots + f29/f0 + fi/f0$

where f0 represents the initial fluorescence reading at 0 minutes, and fi represents the fluorescence reading at time i.

$$\text{Net AUC} = \text{AUCsample} - \text{AUCblank}$$

Where AUCsample is the area under the curve of the sample (sample with fluorescein solution and AAPH), and AUCblank is the area under the curve of the blank (phosphate buffer with fluorescein solution and AAPH).

2.9 Statistical analysis

All experiments were performed in triplicate. The results were carried out as mean value ± standard deviation (SD). Statistical analyses were compared with the Duncan multiple range test, and $p<0.05$ was applied.

3. Results and discussion

3.1 Gamma-aminobutyric acid content

Based on testing the GABA contents of 24 rice samples, it was found that their concentrations ranged from 0.021 to 0.181 mg/g dry weight. The germinated brown rice of the Kam-Noi variety had the highest GABA content. This was followed by the brown rice of the Kam-Noi variety at 0.155 mg/g dry weight, and the germinated brown rice of the Som-Ma-Lee variety at 0.152 mg/g dry weight (Table 1). The GABA content of the Kam-Noi variety examined in this study closely resembled that of the glutinous rice varieties Taichung (0.175 mg/g dry weight) and Niaw Sanpatong (0.193 mg/g dry weight) (Karladee and Suriyong, 2012).

Table 1. GABA content in brown rice, germinated brown rice, and germinated Hang rice.

Samples	GABA content (mg/g dry weight)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	0.113 ± 0.006^{B}	0.102 ± 0.002^{B}	0.034 ± 0.001^{F}
Mali-Daeng	0.021 ± 0.001^{G}	0.117 ± 0.006^{B}	0.063 ± 0.003^{D}
Mali-Bao	0.063 ± 0.004^{D}	0.035 ± 0.003^{F}	0.044 ± 0.001^{F}
Kam-Noi	0.155 ± 0.006^{A}	0.181 ± 0.002^{A}	0.024 ± 0.001^{G}
Som-Ma-Lee	0.094 ± 0.003^{B}	0.152 ± 0.004^{A}	0.086 ± 0.004^{B}
Hom-Thung	0.082 ± 0.001^{C}	0.064 ± 0.004^{D}	0.025 ± 0.004^{G}
Nieow-Daeng	0.022 ± 0.001^{G}	0.022 ± 0.001^{G}	0.027 ± 0.002^{G}
Pong-Aew	0.056 ± 0.005^{E}	0.044 ± 0.001^{F}	0.032 ± 0.001^{F}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

Based on the experiment's findings, germinated brown rice has a higher GABA content. This could be attributed to the production of GABA, an amino acid, during the germination phase (Hong and Kim, 2019). However, when comparing Hang rice to germinated brown rice and brown rice, its GABA content is lower. This outcome may be due to the use of heat during the creation of Hang rice, where the grain is steamed until cooked, dried, and polished to remove husks. The heat involved in this process could potentially impact GABA levels. Additionally, during husk removal, the rice germ containing GABA may also be removed (Suwannatrai *et al.*, 2022). These findings concurred with a study by Jandaruang and Preecharram (2020), who found that cooked rice had lower GABA levels compared to uncooked rice. Together with GABA, other components of germinated rice include campesterol, hydroxybenzoic acid, and α-tocotrienol (Jayadeep and Malleshi, 2011; Kwak *et al.*, 2013; Cho and Lim, 2016).

3.2 Total phenolic compound content

After quantifying the phenolic compounds present in each of the 24 rice samples, it was found that their concentrations ranged from 0.008 to 0.074 mg/g dry weight. The highest concentration of phenolic compounds was observed in Mali-Daeng germinated brown rice, followed by Nieow-Daeng germinated Hang rice (0.073 mg/g dry weight), and Kam-Noi brown rice

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(0.071 mg/g dry weight) (Table 2).

Hang rice exhibits a lower concentration of phenolic compounds compared to brown rice and germinated brown rice. This finding from the experiment could be attributed to specific phenolic compounds decomposing due to heat during the production of Hang rice. These results align with studies by Jandaruang and Preecharram (2020) and Pitiwiwattanakul *et al*. (2011), which demonstrated that heat-treated rice had reduced levels of phenolic compounds. According to Tian *et al*. (2004), most phenolic compounds in rice are water-soluble molecules, such as 6'-O-(E)-feruloylsucrose. Additionally, heat has been shown to decrease the quantity of phenolic compounds in quince fruits (Maghsoudlou *et al.*, 2019).

Table 2. Total phenolic compound content in brown rice, germinated brown rice, and germinated Hang rice.

Samples	Total phenolic content (mg/g dry weight)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	0.029 ± 0.001^{E}	0.019 ± 0.003^{H}	0.010 ± 0.003^{L}
Mali-Daeng	0.035 ± 0.003^{D}	0.074 ± 0.004^{A}	0.033 ± 0.004^{D}
Mali-Bao	0.013 ± 0.003^{J}	0.019 ± 0.001^{H}	0.012 ± 0.001^{K}
Kam-Noi	0.071 ± 0.002^{A}	0.045 ± 0.006^{C}	0.059 ± 0.001^{B}
Som-Ma-Lee	0.026 ± 0.005^{F}	0.025 ± 0.006^{G}	0.010 ± 0.002^{L}
Hom-Thung	0.024 ± 0.003^{G}	0.023 ± 0.003^{H}	0.010 ± 0.002^{L}
Nieow-Daeng	0.063 ± 0.002^{B}	0.039 ± 0.006^{C}	0.073 ± 0.006^{A}
Pong-Aew	0.016 ± 0.001^{I}	0.011 ± 0.001^{M}	0.008 ± 0.001^{M}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

3.3 Anthocyanin content

Based on an examination of the anthocyanin content of samples from four different varieties of colored rice: Chao-Daeng, Mali-Daeng, Kam-Noi, and Nieow-Daeng. There are twelve rice samples altogether. The experiment revealed anthocyanin content ranging from 0.045 to 24.865 mg/L. The brown rice sample from the Kam-Noi variety demonstrated the highest anthocyanin concentration, followed by samples of germinated brown rice (11.370 mg/L) and germinated Hang rice (7.815 mg/L), both also from the Kam-Noi variety (Table 3). In northeastern Thailand, the total anthocyanin concentration ranged from 26-254 mg/100 g across thirty native purple waxy rice cultivars (Phonsakhan and Kong-Ngern, 2015).

Based on the experiment's findings, the sticky rice variety Kam-Noi exhibits a significantly higher anthocyanin content compared to other rice varieties. This may be attributed to Kam-Noi rice's darker purple hue, as it is known that glutinous rice varieties like Kam-Noi tend to have elevated anthocyanin levels compared to non-glutinous rice varieties (Phan *et al.*, 2018). Germinated Hang rice has a lower anthocyanin content. This could be because during the production of Hang rice, the water-soluble phenolic compounds known as anthocyanins are broken down (Khoo *et al.*, 2017).

Table 2. Total phenolic compound content in brown rice, germinated brown rice, and germinated Hang rice.

Samples	Anthocyanin content (mg/L)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	0.325 ± 0.035^{D}	0.550 ± 0.113^{D}	0.215 ± 0.035^{D}
Mali-Daeng	0.045 ± 0.008^{D}	0.110 ± 0.028^{D}	0.045 ± 0.002^{D}
Kam Noi	24.865 ± 0.304^{A}	11.370 ± 0.863^{B}	7.815 ± 1.153^{C}
Nieow-Daeng	0.430 ± 0.057^{D}	0.470 ± 0.041^{D}	0.300 ± 0.141^{D}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

3.4 Protein content

The protein content analysis results were obtained using the Kjeldahl method for all 24 rice samples; their concentrations ranged from 6.090 - 8.380%. The Hom-Thung germinated brown rice sample exhibited the highest protein content, followed closely by the Pong-Aew germinated Hang rice at 7.710%, the germinated Hang rice of the Hom-Thung variety, and the germinated brown rice of the Mali-Bao variety, both registering at 7.500% (Table 4).

Table 4. Protein content analyzed by Kjeldahl method in brown rice, germinated brown rice, and germinated Hang rice.

Samples	Protein content (%)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	6.920 ± 0.110^{GHI}	6.790 ± 0.010^{HIJ}	6.790 ± 0.010^{HIJ}
Mali-Daeng	6.920 ± 0.110^{GHI}	7.250 ± 0.070^{EF}	7.210 ± 0.020^{EF}
Mali-Bao	6.090 ± 0.170^{L}	7.500 ± 0.140^{CD}	6.250 ± 0.070^{KL}
Kam-Noi	6.670 ± 0.210^{J}	7.000 ± 0.140^{GH}	6.630 ± 0.110^{J}
Som-Ma-Lee	6.750 ± 0.070^{IJ}	6.920 ± 0.030^{GHI}	6.380 ± 0.030^{K}
Hom-Thung	7.670 ± 0.030^{BC}	8.380 ± 0.030^{A}	7.500 ± 0.140^{CD}
Nieow-Daeng	7.590 ± 0.140^{BC}	7.250 ± 0.070^{EF}	7.380 ± 0.030^{DE}
Pong-Aew	7.090 ± 0.010^{FG}	7.380 ± 0.070^{DE}	7.710 ± 0.030^{B}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

Interestingly, germinated brown rice typically exhibits a higher protein level compared to regular brown rice. This phenomenon may be attributed to the production of GABA, an amino acid, during the

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germination process (Hong and Kim, 2019). Consequently, the protein content experiences an elevation. Notably, the nitrogen content of GABA, as an amino acid, is also assessed using the Kjeldahl protein analysis method.

When comparing different rice strains, it was observed that each strain has varying protein content, influenced by the specific soil and environmental conditions of its growth. The protein content of the rice studied in this investigation aligns with that of brown rice, falling within the range of 5-11%. Rice is the second most popular source of protein, following carbohydrates. Rice protein is notable for containing important amino acids such as aspartic acid, glutamic acid, and lysine, which are essential for human health (Carcea, 2021).

The amount of protein in rice affects its physicochemical characteristics. Compared to most cereals, rice generally has a lower protein content. It also has very little fat and fiber in it. Nevertheless, rice outperforms other cereals such as oats, wheat, millet, corn, sorghum, and barley in terms of digestible energy and net protein consumption (Mohidem *et al.*, 2022). While certain proteins are present in rice, like globulin and albumin, they do not cause allergic reactions (Amagliani *et al*., 2017).

3.5 Molecular weight of protein rice

The molecular size of proteins was examined using SDS-PAGE. The results revealed that the proteins from brown rice extract samples, Mali-Daeng (lane 3), Kam-Noi (lane 6), and Nieow-Daeng (lane 9), exhibited identical molecular mass sizes, appearing as protein bands with a molecular weight of 14.40 kDa. Similarly, samples Chao-Daeng (lane 2), Pong-Aew (lane 4), Mali-Bao (lane 5), Hom-Thung (lane 7), and Som-Ma-Lee (lane 8) also displayed protein bands, with molecular weights of 14.40, 15.13, 21.87, 25.11, 26.30, 31.62, 36.30, 39.81, 52.48, 57.54 and 69.18 kDA (Figure 2(A))

The protein bands from germinated brown rice extract samples, Mali-Daeng (lane 3), Mali-Bao (lane 5), Kam-Noi (lane 6) and Nieow-Daeng (lane 9) exhibited identical molecular weight at 14.40 kDa. The Chao-Daeng (lane 2), Pong-Aew (lane 4), Hom-Thung (lane 7), and Som-Ma-Lee (lane 8) samples also displayed protein bands, with molecular weights of 14.40, 15.13, 20.89, 25.11, 30.19, 36.30, 53.70, and 63.09 kDa (Figure 2(B)).

The protein bands from germinated Hang rice extracts; Chao-Daeng sample (lane 2), exhibited the protein band at molecular weights of 14.40, 14.45, 20.89, 25.11, 31.62, 37.15, 57.54, and 70.79 kDa. The Mali-Daeng (lane 3) and Mali-Bao (lane 5) samples also displayed protein bands, with molecular weights of 14.40 and 44.66 kDa (Figure 2(C)).

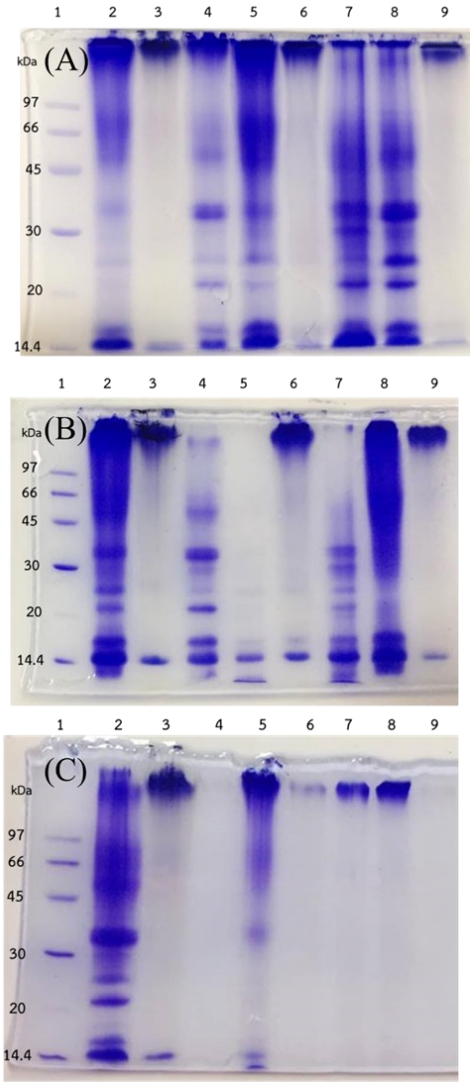

Figure 2. Molecular weight of protein marker and (A) brown rice, (B) germinated brown rice, and (C) germinated Hang rice on 15% SDS-PAGE. Lane 1: standard protein marker, Lane 2: Chao-Daeng, Lane 3: Mali-Daeng, Lane 4: Pong-Aew, Lane 5: Mali-Bao, Lane 6: Kam-Noi, Lane 7: Hom-Thung, Lane 8: Som-Ma-Lee and Lane 9: Nieow-Daeng.

Based on the experimental results, the protein pattern observed in brown rice closely resembles that of germinated brown rice. A protein band was evident on the germinated Hang rice, in contrast to brown rice and germinated brown rice. This phenomenon is attributed to the heating involved in the production process of germinated Hang rice, which likely influences the breakdown of hydrogen bonds within the protein structure. Rice proteins can be categorized into four main groups based on their molecular weight: prolamin, with a molecular weight ranging from 10-53 kDa, glutelin (10-66 kDa), globulin (20-66 kDa), and albumin protein (30-45 kDa). However, when considering proteins derived from rice bran, they will have a range the broad molecular mass sizes. The molecular masses for albumin

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(10-100 kDa), globulin (10-150 kDa), glutelin (33-150 kDa), and prolamin (25-100 kDa) (Jayaprakash *et al.*, 2022). High levels of variability have been reported for rice albumin (Braspaiboon *et al.*, 2020), which includes proteins with molecular weights ranging from 10 to 200 kDa. Interestingly, a 60 kDa glycoprotein and a 16 kDa protein are the main weights. Proteins found in rice are grouped according to their molecular weights, which range from 13 to 110 kDa (Hozzein *et al.*, 2019). Typically, proteins in the prolamin fraction have molecular weights between 10 and 17 kDa. The solubility of the protein albumin in water is one property that aids in its ability to retain water. So, this is crucial for people who have liver cancer and edema.

3.6 Antioxidant activity

Antioxidant activity analysis was conducted using the DPPH technique, yielding IC_{50} values ranging from 15.600 to 275.000 μg/μL. Among the samples tested, Nieow-Daeng germinated Hang rice exhibited the highest antioxidant ability with an IC_{50} value of 15.600 μg/μL, followed by brown rice and germinated brown rice with IC_{50} values of 19.300 μg/μL and 26.000 μg/μL, respectively (Table 5). This suggests that the high phenolic component content in Nieow-Daeng rice may contribute significantly to its superior antioxidant qualities. This aligns with the findings of Butsat and Siriamornpun (2010), who demonstrated that extracts rich in phenolic compounds also possess robust antioxidative qualities. Additionally, both Mali-Daeng and Kam-Noi rice demonstrated strong antioxidant properties as depicted in Table 5. The antioxidant activity of Kam-Noi rice was found to be directly correlated with its quantities of phenolic compounds, GABA, and anthocyanin. Similarly, the antioxidant ability of Mali-Daeng rice showed a direct correlation with its levels of GABA and phenolic compounds. Nieow-Daeng and Kam-Noi are black glutinous rice. Researchers assert that phenolic compounds, γ-orizanol and tocols present in black glutinous rice and germinated brown rice, including p-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, gallic acid, guaiacol, campesterylferulate, tocopherol and tocotrienol, efficiently inhibit free radicals (Cho and Lim, 2016; Pramitasari and Herlina, 2023). The experimental results show that the phenolic compounds have the strongest positive correlation with DPPH antioxidant activity, with a linear correlation coefficient (r) of 0.800.

Anthocyanins, GABA, and phenolic substances represent a group of compounds known for their potent antioxidant capabilities (Shao *et al.*, 2014; Khoo *et al*., 2017; Wu *et al.*, 2022). Anthocyanins exhibit antioxidant activity thanks to the presence of hydroxyl groups, which are capable of donating hydrogen atoms to reactive species (Pramitasari and Herlina, 2023). Phenolic compounds, in their active state, scavenge oxygen and donate electrons, thus exhibiting strong antioxidant properties (Abdel-Hameed, 2009). Their fundamental structural component, an aromatic ring often substituted with a hydroxyl group, enables them to trap free radicals effectively. Consequently, extracts with high phenolic concentrations are more resistant to oxidation.

Assessment of metal chelating ability using the FIC technique revealed an IC_{50} value range of 50.000 to 680.000 μg/μL, as indicated by the results (Table 6). Among the tested samples, Hom-Thung germinated Hang rice demonstrated the highest metal chelating capacity (50.000 μg/μL), followed by brown rice (98.000 μg/μL), and germinated brown rice (140.000 μg/μL). Additionally, Pong-Aew rice exhibited significant effectiveness in chelating metals.

The results highlight the strong affinity of Hom-Thung rice for binding Fe^{2+}. This may be attributed to phenolic compounds possessing either a 7–8 o-dihydroxyphenyl structure or a combination of a 4-keto group and a 3-hydroxyl group, as suggested by Guo *et al*. (2007). Consequently, the capacity to chelate Fe^{2+} is

Table 5. Antioxidant activities by DPPH assay of brown rice, germinated brown rice, and germinated Hang rice.

Samples	IC_{50} (μg/μL)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	87.000±1.270^{J}	77.000±1.760^{K}	183.000±2.330^{D}
Mali-Daeng	62.000±0.990^{M}	32.000±1.410^{P}	45.000±1.690^{O}
Mali-Bao	178.000±0.700^{E}	172.000±0.990^{F}	238.000±0.840^{B}
Kam-Noi	34.000±2.120^{P}	35.000±1.130^{P}	55.000±0.420^{N}
Som-Ma-Lee	135.000±1.270^{H}	152.000±2.400^{G}	210.000±0.990^{C}
Hom-Thung	67.000±1.410^{L}	77.000±2.610^{K}	122.000±0.490^{I}
Nieow-Daeng	19.300±0.910^{R}	26.000±0.210^{Q}	15.600±0.910^{S}
Pong-Aew	183.500±2.120^{D}	275.000±1.060^{A}	273.000±0.700^{A}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

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Table 6. Antioxidant activities by FIC assay of brown rice, germinated brown rice, and germinated Hang rice.

Samples	IC_{50} (μg/μL)		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	434.500±0.560^{J}	626.000±1.410^{C}	526.000±0.560^{H}
Mali-Daeng	576.000±0.700^{F}	580.000±0.630^{E}	680.000±0.280^{A}
Mali-Bao	318.000±0.770^{M}	276.000±1.690^{N}	420.000±0.770^{K}
Kam-Noi	539.000±1.130^{G}	630.000±2.750^{B}	230.000±0.990^{P}
Som-Ma-Lee	586.000±1.200^{D}	508.000±1.620^{I}	260.000±0.840^{O}
Hom-Thung	98.000±0.700^{W}	140.000±0.630^{U}	50.000±0.770^{X}
Nieow-Daeng	362.000±0.070^{L}	196.000±1.130^{S}	186.000±0.990^{T}
Pong-Aew	126.000±0.990^{V}	224.000±0.700^{Q}	204.400±0 .140^{R}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

influenced by the specific phenolic structure as well as the arrangement and quantity of hydroxyl groups.

The analysis results of the 24 rice samples' ability to inhibit peroxyl radicals (ROO•) showed an ORAC value range between 11.310 and 12.960 (Table 7). Among these samples, Hom-Thung brown rice exhibited the strongest defense against free radicals. Following closely were Kam-Noi germinated brown rice and Mali-Daeng brown rice, as illustrated in Table 7, with ORAC values of 12.760 and 12.700, respectively. The comparison among the antioxidant capacities of the 24 rice samples did not reveal significant variations ($p<0.05$) between them.

The ORAC method is based on hydrogen atom transfer and is commonly employed to evaluate the antioxidant activity of foods, plant extracts, and fruit juices. A high ORAC value indicates robust peroxyl antioxidant capabilities in a substance.

Table 7. Antioxidant activities by ORAC assay of brown rice, germinated brown rice, and germinated Hang rice.

Samples	ORAC value		
	Brown rice	Germinated brown rice	Germinated Hang rice
Chao-Daeng	11.960±0.240^{A}	12.580±0.870^{A}	12.570±0.730^{A}
Mali-Daeng	12.700±0.430^{A}	12.020±0.140^{A}	11.880±0.620^{A}
Mali-Bao	12.130±0.030^{A}	12.550±0.720^{A}	11.910±1.280^{A}
Kam-Noi	12.160±1.180^{A}	12.760±0.500^{A}	12.530±1.550^{A}
Som-Ma-Lee	12.560±0.140^{A}	12.470±0.040^{A}	12.400±1.510^{A}
Hom-Thung	12.960±1.400^{A}	12.210±1.390^{A}	12.360±0.990^{A}
Nieow-Daeng	12.230±0.310^{A}	11.310±1.380^{A}	12.520±0.840^{A}
Pong-Aew	12.100±0.120^{A}	12.270±0.040^{A}	11.610±0.520^{A}

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

Based on the data, both brown rice and germinated brown rice show a trend of high concentrations of bioactive compounds and strong biological activities.

4. Conclusion

Different rice varieties exhibit varying concentrations of bioactive compounds and antioxidant properties. Pigmented rice varieties generally have higher levels of antioxidant activity and bioactive compounds compared to white rice. An example is Kam-Noi, a deep purple rice rich in anthocyanins and GABA. Mali-Daeng is notable for its diverse total phenolic compounds. Strong antioxidant properties are observed in Mali-Daeng, Kam-Noi, and Nieow-Daeng rice types. Additionally, the levels of bioactive compounds and antioxidants vary based on rice processing methods, such as brown rice, germinated brown rice, and germinated Hang rice. However, all rice varieties are also a nutrient-rich source of easily digestible protein.

Conflict of interest

The authors declare no conflict of interest.

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