Original Article

Identification of Nutraceuticals and Antioxidant Activity in Low-Cost Broken Rice Varieties

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Abstract

Rice grains are the main crops consumed by more than half of the world's population. White rice contains carbohydrates, while pigmented rice varieties are more nutritious, with bioactive compounds that can potentially prevent pro-inflammatory diseases like diabetes and hypertension. Broken rice is produced in large quantities as a cheap by-product of milling; however, its nutraceutical contents have not been identified. Therefore, the research aimed to examine four low-cost broken rice varieties' color values and bioactive compound contents. Kiaw-Ngu glutinous rice (white rice) and brown Jasmine rice 105 were used as control, while Riceberry rice and Sangyod rice represented pigmented rice varieties. Color values were analyzed using the RHS color chart and the CIE L*a*b* color space system. Antioxidant activity was measured using DPPH assay, while total phenolic compounds were determined by the Folin–Ciocalteu's method. Water-soluble vitamins (B1, 2, and 6) and fat-soluble vitamins (E and β-carotene) were determined using HPLC. The analyzed results illustrated that the Riceberry rice had the most potent antioxidant activity (83.45%), followed by Sangyod rice, brown Jasmine rice 105, and Kiaw-Ngu glutinous rice, respectively. Similarly, Riceberry rice contained the highest level of total phenolic acid (236.98 mg gallic acid equivalent (GAE)/g). Vitamin B1 level is highest in the Riceberry rice, while vitamin B2 level was comparably highest in the Riceberry rice and Sangyod rice. However, vitamin B6 was not detectable in any strains of the rice varieties. The Riceberry rice and Sangyod rice also contained vitamin E and β-carotene. In conclusion, pigmented rice grains possess antioxidant activity, phenolic acids, vitamins B1, B2, E, and β-carotene.

Keywords: pigmented rice, antioxidant, phenolic acids, vitamins.

INTRODUCTION

Nowadays, advanced biotechnology has been utilized as a tool for making improvements in rice quality (Biswal et al., 2017). As a result, Thailand is ranked the sixth-largest country worldwide for rice cultivation (Arunrat et al., 2022). Chachoengsao, a province in Thailand, is a large area producing rice varieties, including Kiaw-Ngu glutinous rice (white rice), brown Jasmine rice 105, Riceberry rice, and Sangyod rice. White rice grains are produced from two-step milling (dehusking and polishing) to remove the husk, bran, and germ parts, granting the white part a primary source of carbohydrates. On the other hand, unpolished rice grains are only dehusked and thus still contain the innermost white rice, germ, and bran layers (Shafie & Esa, 2017).

The rice bran layer is an edible part comprising pericarp, aleurone, and subaleurone fractions (Gul et al., 2015). The pigmented (black, brown, orange, purple, and red) rice grains contain all the nutrients in the aleurone layer, making them more nutritious than their white rice counterpart (Mbanjo et al., 2020).

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Pigmented rice grains contain nutraceuticals like total phenolic acids, vitamins, and antioxidative substances in addition to carbohydrates and proteins. These nutraceuticals fight against severe diseases like cancer, hypertension, and inflammation (Chen et al., 2012; Hartati et al., 2017; Jan-On et al., 2021). Black rice is high in proteins, fat, and fibers, while red rice varieties are rich sources of minerals consisting of iron and zinc. Additionally, black and red rice grains acquire their colors from phenolic pigments, having antioxidant capacities and other health benefits. Brown rice grains contain vitamins B1, B2, B6, and E, carotenoids, and niacin (Rathna Priya et al., 2019). Broken rice is produced in large quantities (30%) during the husk removal and polishing of the rice grains, and it is then sold as a cheap by-product of milling (Sapna et al., 2019). This study aimed to identify the bioactive compounds in the four low-price broken rice varieties from Ban Pho district, Chachoengsao. Since the broken rice varieties might contain a respectable amount of total phenolic content and antioxidant activity, we hypothesized that they would be valuable for further application in the food industry.

Materials and Methods

Experimental design and sample preparation

This study was a completely randomized design (CRD). The four varieties of broken rice (Kiaw-Ngu glutinous rice, brown Jasmine rice 105, Riceberry rice, and Sangyod rice) were purchased from Ban Pho District, Chachoengsao Province farmers. This study was a sub-project of the in vivo study and therefore has been approved by the Human Research Ethics Committee, Rangsit University, COA. NO, RSUER82021-106. The procedure was modified from a previous study (Tyagi et al., 2022). All rice grains (100 g powder) were dry-ground using hammer milling and filtered with a 200-Mesh strainer at room temperature. The dry-ground rice samples are illustrated in Figure 1.

Figure 1. The dry-ground rice samples. A, Kiaw-Ngu glutinous rice; B, brown Jasmine rice 105; C, Riceberry rice; and D, Sangyod rice.

Measurements of colors, moisture, and water activity of rice varieties

The colors of rice grains were measured by two methods. First, the colors were recorded according to the Royal Horticultural Society (RHS) color chart, the standard reference used by horticulturists worldwide (Chart, 2015). Secondly, we used the CIE L*a*b* color space system. The lightness (L*), red/green is (a*), and yellow/blue is (b*) values of color were measured by colorimeter (Minolta CR 400, Germany) [12, 13]. In addition, moisture and water activity (aw) were measured using the EZ-200, Japan, according to the AOAC 2000.

Antioxidant activity measurements

Rice extracts' antioxidant activity was analyzed using a 2,2-diphenylpicrylhydrazyl (DPPH) assay (Bakir et al., 2018). The color of the DPPH radical solution was purple, with an absorbance range from 515 to 517 nm. Therefore, with an increasing antioxidant concentration, the DPPH color turns lighter and can be measured by a spectrophotometer. The 15-g rice samples were weighed, added 30 ml of methanol was set aside for 24 hours, filtered with filter paper No. 1, diluted the filtered solution with 100 ml of purified water, and 1000 µl of the tested samples were used. DPPH solution (0.1 mM, 3 ml) was pipetted into each Trolox concentration and left in the darkroom for 30 min. Serial diluting concentrations of Trolox (31.25-500 mM) were used as a standard. The absorbent spectrum at 515 nm was determined by spectrophotometry machine (Shimadzu, UV-1601, Japan), respectively, to the blank. The amount of antioxidants needed to lessen DPPH concentration is the percentage of free radical scavenger activity (% inhibition). The following equation was employed to compute the percentage of the radical scavenger activity.

$$\% \text{ inhibition} = [(A0 - A1)/A0] \times 100$$

A0 control solution = concentration (no antioxidant) and A1 = sample solution concentrations (when antioxidant existed) (Huang et al., 2005).

Total phenolic content measurements

The methanol-extract rice samples were measured for the total phenolic contents using the Folin–Ciocalteu (Karagözler et al., 2008). Gradient concentrations of gallic acid (0–300 mg/ml) were used for standard curve plotting. Gallic acid standard solution (400 µl) was pipetted into 2 ml Folin–Ciocalteu solution (Loba cnemie Pty, India), homogenized and inoculated at room temperature for 5 min. Subsequently, 1.5 ml of 7.5% Na2CO3 v/v was added and incubated for 0.5 h at room temperature. The UV spectrophotometry method was exploited to determine the absorbance of the mixed solution at 765 nm (Shimadza, UV-1601, Japan). We measured the total phenolic contents in 400 µl samples sample by calculating it against the standard curve. All were measured three times. Finally, the regression equation of the standard curve was plotted to calculate the total phenolic concentrations in the samples. The results show mg of gallic acid equivalent (GAE)/100 g dry weight.

Determination of vitamin contents

The water-soluble vitamins (B1, B2, and B6) and fat-soluble vitamins (E and β-carotene) were determined using the HPLC system as previously described in the protocols of the AOAC International, Official Methods of Analysis (Horwitz & Latimer Jr, 2005; Sami et al., 2014). All measurements were performed by the Asia Medical and Agricultural Laboratory and Research Center (AMARC), Thailand, a laboratory recognized by local and global food, medical, and agricultural standard systems (https://amarc.co.th/en).

Statistical analysis

Data were presented as means±SD. Multiple group analysis was applied by one-way ANOVA. Significant differences among means were analyzed by Duncan's multiple range test (DMRT) with a significance at $P < 0.05$ (Minitab Pty Ltd, Sydney, NSW, Australia). Pearson's correlation was used to identify a relationship between two continuous data.

Results and Discussion

Colors of rice varieties

It is well-known that pigments in plants are associated with their phytonutrient contents. Over the past decades, many health organizations, such as the United States Department of Agriculture (USDA) Food and Nutrition Service, the American Heart Association, and the American Institute for Cancer Research, have promoted healthy food-based colors (Minich, 2019). Results showed that the color value of Kiaw-Ngu glutinous rice was a white group in the white A range (Table 1). The brown Jasmine rice 105 had the color value in the yellow-white group (pale yellow). The Riceberry rice appeared to be a deep shade of reddish black with bare eyes. As for the Sangyod rice, it was in the yellow-orange group. These distinct colors designate their nutritional constituents. The moisture contents of all rice varieties were 4.96-5.32% and aw of 0.15-0.16.

Table 1 Physical properties of rice varieties.

Rice sample	RHS color	L	a	b	a_w	Moisture content (%)
Kiaw-Ngu glutinous rice	White group in white A	64.78±0.04	1.90±0.02	7.43±0.05	0.15±0.02	5.32±0.04
Brown Jasmine rice 105	Yellow-white group in pale yellow	51.78±0.08	1.89±0.05	9.90±0.02	0.16±0.02	5.09±0.02
Riceberry rice	Red group in deep red	37.67±0.03*	7.94±0.05*	23.01±0.10*	0.15±0.01^{ns}	4.96±0.05 ^{ns}
Sangyod rice	Yellow-orange group in vivid yellow	56.09±0.05*	5.11±0.03*	15.01±0.10*	0.16±0.02 ^{ns}	5.12±0.05 ^{ns}

Colors were measured according to the RHS color chart and the CIE L*a*b* color system. aw, water activity. *, $P < 0.05$ compared to Kiaw-Ngu glutinous and Brown Jasmine 105 rice. NS, not significant.

Profiles of vitamin contents in extracts of rice varieties

Nutrient densities across colorful plant products have been proposed. The dark-green vegetable leaves contain a high amount of vitamin K (Sim et al., 2020). On the other hand, red and orange vegetables are documented for their carotenoids (α-carotene, β-carotene, cryptoxanthin, lutein, and lycopene) (Carazo et al., 2021). The profiles of vitamin contents in extracts of rice varieties are shown in Figure 2. Among four rice varieties, the Riceberry rice had the highest concentration of vitamin B1 (0.24 mg/100 g), followed by Sangyod rice (0.21 mg/100 g), Brown Jasmine rice 105 (0.19 mg/100 g), and Kiaw-Ngu glutinous rice (0.04 mg/100 g) (Figure 2A). Kennedy and Burlingame also reported that the content of vitamin B1 in 79 rice varieties was between 0.117–1.74 mg/100 g (Kennedy & Burlingame, 2003). In the present study, vitamin B1 levels in the three colored Thai rice varieties were in the previously reported range. Interestingly, the Riceberry rice and Sangyod rice had the comparably highest vitamin B2 (0.81 and 0.80 mg/100 g) (Figure 2B). The previous study found that vitamin B2 in 79 rice varieties was between 0.011-0.403 mg% (Kennedy & Burlingame, 2003). This finding signified that the Riceberry rice and Sangyod rice had a doubled vitamin B2 compared to other types of rice. In its purified solid form, it is a water-soluble yellow-orange powder (Pinto & Zempleni, 2016). Hence, it is unsurprising that vitamin B2 mainly existed in Riceberry rice and Sangyod rice. Vitamin B6 was not detectable in our rice varieties. Vitamin B6 has been previously found in rice but at a superficial level, i.e., 1.2-3.9 µg/g. This might explain why we could not detect the presence of vitamin B6 in our rice varieties. The Riceberry rice also had the highest level of vitamin E (0.370 mg/100 g), followed by the Sangyod rice (0.09 mg/100 g). Notably, the brown Jasmine rice 105 (<0.02 mg/100 g).and Kiaw-Ngu glutinous rice contained no vitamin E (Figure 2C). Zubair and colleagues showed that wide Pakistani Basmati rice varieties had vitamin E, especially those without hull removal and extensive milling process (Zubair et al., 2012). The reason for undetectable vitamin E in brown Jasmine rice 105 is not known. Finally, the Riceberry rice had the highest level of β-carotene (0.020 mg/100 g), while the Sangyod rice contained 0.013 mg/100 g. Again, the brown Jasmine rice 105 and Kiaw-Ngu glutinous rice had no β-carotene (Figure 2D). Orange fruits such as mandarins, oranges, tangerines, papaya, peaches, and persimmons contain β-carotene (Minich, 2019). Thus, it is explainable that the Riceberry rice and Sangyod rice with the yellow-orange-red spectra had β-carotene.

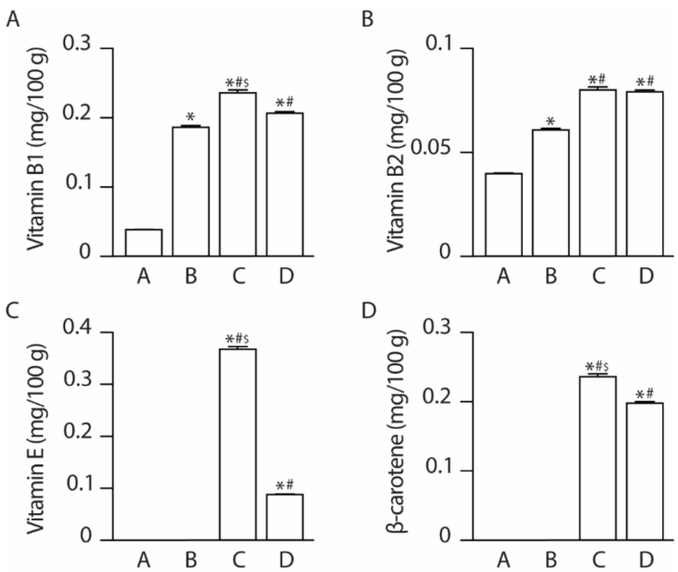

Figure 2. Profiles of vitamin contents in 4 rice varieties. X-axis: A, Kiaw-Ngu glutinous rice; B, brown Jasmine rice 105; C, Riceberry rice; D, Sangyod rice. *, P < 0.05 compared to A; #, P < 0.05 compared to B; $, P < 0.05 compared to D.

Antioxidant activity in extracts of rice varieties

Black and brown rice contains phenolic chemicals and antioxidants associated with several health advantages, including prevention or reduction of type 2 diabetes, pro-inflammatory process, heart disease, cancer, neurological disorders, hypertension, and obesity (Ito & Lacerda, 2019). The DPPH assays exhibited that the Riceberry rice extract contained the highest level of antioxidant activity 83.45 %Trolox equivalent, followed by Sangyod rice (75.61%), Brown Jasmine rice 105 (70.10%), and Kiaw-Ngu glutinous rice (56.09%), respectively (Table 2). It has been demonstrated that anthocyanins are potent antioxidants (Tyagi et al., 2022). However, we did not investigate the presence of anthocyanin in this study. Further study should cover more nutraceuticals.

Table 2 Antioxidant activity in extracts of rice varieties

Rice sample	Antioxidant activity (%Trolox equivalent)
Kiaw-Ngu glutinous rice	56.09 ± 0.08^d
Brown Jasmine rice 105	70.10 ± 0.05^c
Riceberry rice	83.45 ± 0.07^a
Sangyod rice	75.61 ± 0.03^b

Values are mean ± SD. Different superscript small letters in the same column indicate significant differences (P < 0.05).

Total phenolic acid contents in extracts of rice varieties

Phenolic compounds have been used to treat common human illnesses like hypertension, metabolic issues, inflammatory infections, and neurodegenerative disorders (Rahman et al., 2021). Phenolic compounds can block enzymes linked to the development of human diseases. For example, it has been used to treat hypertension by blocking the angiotensin-converting enzyme (ACE) (Hugel et al., 2016). The contents of total phenolic acid in rice variety extracts were also measured. Results showed that the Riceberry rice extract contained the highest concentration of entire phenolic acid contents (236.98 mg GAE/g), followed by Sangyod rice (202.65 mg GAE/g), and Brown Jasmine rice 105 (190.23 mg GAE/g), respectively. Meanwhile, the white Kiaw-Ngu glutinous rice contained the lowest total phenolic acid content (135.14 mg GAE/g) (Table 3). In addition, the total phenolic contents were significantly correlated with the antioxidant activity (P < 0.05 with R2 linear = 0.994, Figure 3). Other nutraceuticals were also ubiquitously present in rice. For example, GABA, amino acids, ferulic acid, p-coumaric acid, quercetin, and ascorbic acid were identified in germinated brown rice (Tyagi et al., 2022). Moreover, our study did not investigate proanthocyanidins and anthocyanins, major flavonoids in pigmented rice (Mbanjo et al., 2020). It has been reported that hammer milling preserved total phenolic content better than plate milling (Sapna et al., 2019). The effects of heat on nutraceuticals' availability should also be considered. Oryzanol, a nutraceutical primarily present in rice bran, was also reduced by rice toasting and increased by direct steaming (Sapna et al., 2019). Thus, for further applications, hammer milling, and conventional steaming might be suitable for rice cooking.

Table 3 Total phenolic acid contents in extracts of rice varieties

Rice sample	Total phenolic acid (mg GAE/g)
Kiaw-Ngu glutinous rice	135.14 ± 0.05^d
Brown Jasmine rice 105	190.23 ± 0.08^c
Riceberry rice	236.98 ± 0.05^a
Sangyod rice	202.65 ± 0.09^b

Values are mean ± SD. Different superscript small letters in the same column indicate significant differences (P < 0.05).

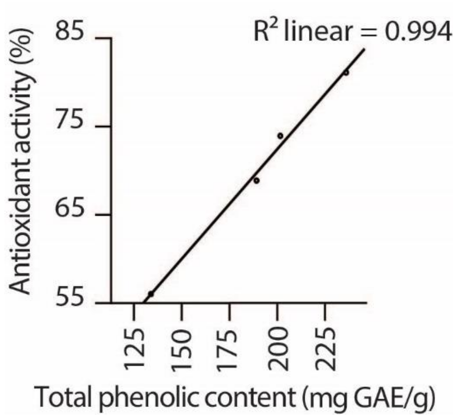

Figure 3. Pearson's correlation between the total phenolic contents and the antioxidant activity

Conclusion

In conclusion, the four varieties of pigmented broken rice grains (Kiaw-Ngu glutinous rice, brown Jasmine rice 105, Riceberry rice, and Sangyod rice) differentially possess antioxidant activity due to their different colors, phenolic acids, vitamins B1, B2, E, and β-carotene. It can be highlighted that Riceberry rice is the most nutritious one and should be encouraged to be consumed more by Thai and other people worldwide. The limitation of this study was that we did not measure concentrations of other nutraceuticals, such as tannins and anthocyanins, which also confer antioxidant activity. Therefore, we considered it a negative result since other potential nutraceuticals have not been investigated. Further study is required to explore more in vitro and in vivo before applying these pigmented rice varieties in the food for the health industry.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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