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Correlation of Antioxidant Activity and Phytochemical Profile in Brown Rice and Brown Rice Products

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Abstract

Brown rice and brown rice products (brown rice, germinated brown rice, dry germinated brown rice, germinated brown rice noodle and dry germinated brown rice noodle) were studied for their total phenolic, total flavonoid, γ-aminobutyric acid (GABA) content and antioxidant activity by DPPH and FRAP assay. The results indicate that the brown rice extract shows the highest on total phenolic contents (0.52±0.03 mg GAE/g of fresh weight), followed by germinated brown rice, germinated brown rice noodle, and dry germinated brown rice noodle, respectively. The total flavonoid content is only found in brown rice (0.35±0.03 mg quercetin/100 g sample). In GABA content, the germinate brown rice shows the highest on GABA value (75.02±0.52 mg GABA/g of fresh weight), followed by germinate brown rice noodle, dry germinate brown rice noodle, and brown rice, respectively. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (IC_{50}) were determined for the antioxidant activity, germinated brown rice shows the highest DPPH (IC_{50}; 27.2±4.3 mg/mL), followed by brown rice, and dry germinated brown rice, respectively. Brown rice shows the highest FRAP (5.95±8.44 mM Fe (II) /g of fresh weight), followed by germinate brown rice, germinate brown rice noodle, and dry germinate brown rice noodle, respectively.

Introduction

Rice is the seed of the grass species *Oryza sativa* L. that originated in India, Thailand, and southern China. It is roughly divided into two types, indica and japonica. It is a common crop and a very important food source of the world. Rice is considered one of natural resources that contains a large phytonutrients and biologically active ingredients such as oryzanols, vitamin E, vitamin B complex, carotenoids and phenolic compounds (Carlos et al., 2007; Okarter et al., 2010; Watchararparpaiboon et al., 2012; Liu, 2007). Several studies have reported on the chemical characterization and quantification of the chemicals in rice and health benefits such as enhancement of the immune system, lowers heart disease, cardiovascular disease, glycemic control, diabetes, and cancer prevention (Hsu et al., 2008; Okarter & Liu, 2010; Jiang et al., 2016). Brown rice is an unpolished whole grain that is produced by removing the husk and its color may be light brown, reddish, purplish or black. It is a whole grain that contains the bran and germ. The grain provides nutritious components, such as dietary fiber, minerals, vitamins,

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γ-aminobutyric acid (GABA) and antioxidants (Gong et al., 2017). Many studies have reported on the antioxidant activity and phytochemical compounds in rice and rice products such as seed, bran, rice flowers, brown rice and germinated brown rice, etc. (Nakornriab & Krasaerep, 2018; Er et al., 2017a; Inket & Phugan, 2017; Hiran et al., 2015; Kerdchoechuen et al., 2013). As a result, it has been reported that the consumption of brown rice can reduce the cardiovascular diseases, type II diabetes, obesity and cancer (Wu et al., 2013). Recently, germinated brown rice has been noted as one of the most interesting germinated cereal products. Important bioactive compounds in germinated brown rice (GBR) shows a significant improvement after germination, for example, γ-aminobutyric acid, dietary fiber, ferulic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol and prolylendopeptidase inhibitor (Kayahara et al., 2000; Wu, et al., 2013). Accordingly, GBR has been reported to exert many positive effects on the reduction in chronic diseases, hyperlipidemia and hypertension (Hyun et al., 2012). GABA is an important inhibitory neurotransmitter in the brain and spinal cord of mammals and shows a series of functions, such as the inhibition of cancer cell proliferation and stimulation of cancer cell apoptosis, regulation of blood pressure and blood holesterol, heart rate, and alleviation of pain and anxiety (Liao et al., 2013; Zhang et al., 2014).

Recently, Thai rice varieties have been growing in popularity and are demanding higher prices in the Asian rice market. The cultivars of rice, such as brown rice, acknowledge the high amounts of nutrients and a large number of biologically active phytochemicals which are widely recognized as being capable of maintaining human health for people consumption, especially in Southeastern Asia (Hu et al., 2003). The Thai Ministry of Agriculture expects rice production to yield around 25 million tonnes of paddy rice in the 2016-2017 crop year, down from 27.06 million tonnes in 2015-2016 (Chris & Supunnabul, 2016; Simon, 2016). So, the processing of rice for export should be an alternative way to increase income for farmers. Therefore, the research was aimed to investigate the correlation of antioxidant activity and phytochemical profile in brown rice and products; brown rice, germinated brown rice, dry germinated brown rice, germinated brown rice noodle and dry germinated brown rice noodle. The phytochemical profiles include the contents of phenolics, flavonoids, and gamma-aminobutyric acid contents.

Materials and methods

1. Plant materials

Whole rice in this study was non-glutinous white rice (Khao Leuang Patew). Khao Leuang Patew paddy rice was grown in fieldsin Mahasarakham Province, Thailand, on February 2015. All rice products such as brown rice, germinated brown rice, dry germinated brown rice, germinated brown rice noodle and dry germinated brown rice noodle. Brown rice was produced by mechanically removing the husk of paddy rice. Then, the germinated brown rice was produced by soaking brown rice grains in water for 24 hours at room temperature. The water was changed every 6 hours in order to minimize microbial growth. Germinated brown rice noodle and dry germinated brown rice noodle are particularly valued in local markets. All reagents include Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl, gallic acid, sodium carbonate, vitamin E, butylated hydroxyanisole, and all solvents (HPLC grade) were purchased from Fluka (Switzerland)

2. Extraction

The brown rice, germinated brown rice, and germinated brown rice noodle were dried in oven at 50ºC until their moisture contents were reduced to approximately 15% (dw). After that, all brown rice and their products were ground to a fine powder in a mechanical blender and sieved (100 mesh) to a uniform size. All sample powders (5.0 g) were extracted by methanol containing 1.0% HCl (3x100 mL) at room temperature with intermittent shaking for 60 minutes. The extraction was repeated three times until complete extraction. The supernatants were combined and filtered through a 0.45 µm Nylon membrane filter. After that, the supernatants were then slowly concentrated under reduced pressure below 40°C on a rotary evaporator to yield the crude extracts. All crude extracts were stored at 4°C in storage vials for determination of phytochemicals and antioxidant activities.

3. Phytochemical screening

Phytochemical screening of crude extracts from brown rice and their products were carried out on the three extracts, total phenolic flavonoids and GABA contents.

3.1 Total phenolic contents

The total phenolic contents of crude extracts from brown rice and their products were determined by spectrophotometric method using Folin-Ciocalteu's phenol

reagent, as described earlier with some modifications by Krasaetep (2012). The crude extracts from brown rice and their products (0.5 mL) were placed in a test tube and were diluted to 5.0 mL with a glass of distilled water. 10% Folin–Ciocalteu's phenol reagent (5.0 mL) was added, and the contents of the test tube were mixed thoroughly. After 15minutes, 5 mL of 10% sodium carbonate solution was added, and the mixture was allowed to stand for 1 hour with intermittent shaking. The absorbance of the blue color was measured in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada) at 750 nm. The concentration of total phenolic compounds was determined using the gallic acid equation (mg of gallic acid equivalent/g of fresh weight) obtained from the standard gallic acid calibration curve. For this purpose, a standard calibration curve was prepared using different concentrations of gallic acid in methanol (20-180 µg/mL). A linear calibration curve of gallic acid resulted with a linear regression equation of calibration curve (Y=1.3496X-0.0063, R^2 =0.9992). This experiment was carried out three times, and the results were averaged for the different fractions in the crude extracts.

3.2 Total flavonoid contents

The total flavonoid contents of crude extracts from brown rice and their products were determined by spectrophotometric method using the aluminium chloride colorimetric method, as described earlier with some modifications by Krasaetep (2012). Pipette crude extracts (250 µL) was placed in a test tube, mixed with 1.25 mL of distilled water and then 75.0 µL of 5% $NaNO_2$ solution and shaken well. Then set aside at room temperature for 5 minutes, then add 150 µL of 10% $AlCl_3$, and shaken well and the mixture was allowed to stand for 6 minutes. Then, 500 µL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 minutes, and absorbance was measured in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada) at 510 nm. The concentration of total flavonoids content was determined using the quercetin equation (mg Quercetin/100 g of fresh weight) obtained from the standard quercetin calibration curve. For this purpose, a standard calibration curve was prepared using different concentrations of quercetin in methanol (0.2-1.2 mg/ml). A linear calibration curve of quercetin resulted with a linear regression equation of calibration curve (Y=0.3405X-0.0035, R_2 =0.9992). This experiment was carried out three times, and the results were averaged for the different fractions in the crude extracts.

3.3 γ-Aminobutyric acid (GABA) content

The GABA content of crude extracts from brown rice and their products were obtained according to the method by Komatsuzaki et al. (2017). Briefly, 0.06 g/mL of extract from brown rice and their products were placed in a test tube with 0.4 mL borate buffer (0.2 M boric acid and 0.2 M sodium borate, pH 9) and 2 mL phenol reagent (6%). After mixing thoroughly and cooling in ice water for 10 minutes, 0.8 mL of 7.5% sodium hypochlorite reagent was added. The test tube was then shaken vigorously in ice water for 10 minutes and placed in a boiling water bath for 5 minutes. It was then immediately cooled by immersion in ice water for 5 minutes. The absorbance of the colorless was measured in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada) at 630 nm. The concentration of GABA content was determined using the γ-aminobutyric acid equation (mg GABA/g of fresh weight) obtained from the standard γ-aminobutyric acid calibration curve. For this purpose, a standard calibration curve was prepared using different concentrations of GABA in methanol (0.1-1.1 mg/ml). A linear calibration curve of GABA resulted with a linear regression equation of calibration curve (Y=1.0667X-0.025, R2 =0.9981). This experiment was carried out three times, and the results were averaged for the different fractions in the crude extracts.

4. Antioxidant activities

Antioxidant activities of crude extracts from brown rice and their products were carried out on the two extracts, the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and Ferric Reducing Antioxidant Power (FRAP) assay.

4.1 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The radical scavenging activity of crude extracts from brown rice and their products were measured using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described earlier with some modifications by Krasaetep (2012). The extract from brown rice and their products (0.01-10.0 mg/mL) 1 mL. were placed in a test tube, mixed with 3.0 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol. The mixture was shaken vigorously and was left to stand for 30 minutes at room temperature in the dark. The absorbance was measured in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada) at 517 nm. The DPPH radical

scavenging activities of crude extracts were expressed as μM of trolox equivalents (TE)/g of fresh weight using a standard curve of trolox. For this purpose, a standard calibration curve was prepared using different concentrations of trolox in methanol (5-40 mg/mL). The control reaction contained all reagents except for the crude samples. The radical scavenging effect was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control at 517 nm, and A_s is the absorbance of the extract/standard at 517 nm.

This experiment was repeated thrice, and the results were averaged for the different fractions in the crude extracts. The radical scavenging activity of crude extract from rice products were reported as IC_{50} value. IC_{50} value is the concentration of the sample required to inhibit 50% of radical. They were calculated using the dose inhibition curve in linear range by plotting absorbance against the corresponding sample concentration.

4.2 Ferric Reducing Antioxidant Power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was performed according to the methods by Xiao et al. (2015) with slightly modification. The FRAP reagent was freshly prepared by adding 10 mM of 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ) (dissolved in 40 mM of HCl), 20 mM of $FeCl_3$ in water and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. The crude extracts from brown rice and their products 10 mg/mL (100 μL) were mixed with 3 mL FRAP reagent in test tubes, mixed with 300 μL of distilled water and undergoes vortex. Both samples and blank were incubated for 4 minutes at 37°C. The absorbance was measured in a Shimadzu UV-2101PC spectrophotometer (Bio-Tek Instrument INC, Canada) at 593 nm. The values obtained were expressed as mM of ferrous equivalent Fe (II)/g of fresh weight using a standard curve of solution of $FeSO_4.7H_2O$. For this purpose, a standard calibration curve was prepared using different concentrations of solution of $FeSO_4.7H_2O$ in methanol (25-125 μg/ml).

5. Statistical analysis

Results obtained were reported as mean ± SD of triplicate measurements. One-way ANOVA and significance differences for multiple comparisons were determined by Turkey's Honest Significant Difference (HSD) at 0.05 probability level.

Results and discussion

1. Phytochemical screening

The average quantity of the phytochemical screening such as total phenolic, total flavonoid and GABA contents of brown rice and their products are shown in Table 1.

Table 1 Total phenolic, Total flavonoid and GABA contents of brown rice and their products (n=3)

Rice products	Total phenolic (mg GAE/g of fresh weight)	Total flavonoid (mg quercetin/100 g of fresh weight)	GABA content (mg GABA/g of fresh weight)
Brown rice	105 ±84.86	16.6±5.2	30±26
Germinated brown rice	39±23	4.4±2.1	195±92
Dry germinated brown rice	23±20	ND	97±44
Germinated brown rice noodle	34±4.3	ND	82±32
Dry germinated brown rice noodle	1.1±2.5	ND	25±11

Remark: ND is not detected.

From Table 1, the total phenolic content (TPC) was measured by Folin-Ciocalteu reagent method using gallic acid as the standard. Brown rice shows the highest amount of TPC (105±84.86 mg GAE/g of fresh weight), while the dry germinated brown rice noodle shows the lowest (1.1±2.5 mg GAE/g of fresh weight). Total flavonoids content (TFC) was determined by the aluminium chloride colorimetric method using quercetin as the standard. The amount of TFC found in brown rice and germinated brown rice (16.6±5.2 and 4.4±2.1 mg quercetin/100 g sample, respectively). Determination of γ-aminobutyric acid content used GABA as the standard. The amount of GABA was in the range of 25±11-195±92 mg GABA/g of fresh weight. The germinated brown rice extract shows the highest amount of GABA, while the dry germinated brown rice noodle shows the lowest. The phytochemical reference of brown rice and their products reveals the GABA and phenolic contents are present in all extracts; total flavonoid is found only in brown rice and germinated brown rice. It suggests that brown rice and their products lack flavonoid compounds responsible for red, purple and blue pigmentation. The TPC and TFC in this study significantly decreased after processed BR products. This could suggest that the rice products composed of color pigment which are the main compounds that include phenolic acids, flavonoids and tannins for antioxidant activity. On the other hand, environment

conditions such as temperature, light, moisture or water may have an effect on the composition of compounds. Other researchers have reported brown rice has TPC 70.3 mg GAE/100g of dry weight (Benzie & Strain, 1996), brown rice from China has TPC 41.70-52.82 mg GAE / 100g of dry weight (Moongngarm & Saetung, 2010), or brown rice from India has TPC 190.75mg of gallic acid equivalent/g of rice (Yanyan et al., 2013; Mithu et al., 2008). The TPC and TFC of eight Chinese varieties of brown rice ranged from 72.45 to 120.13 mg of gallic acid equiv./100 g and 75.90 to 112.03 mg quercetin/100 g sample, respectively (Er et al., 2017b). For GABA contents, the germinated brown rice showed the highest GABA content (195±92 mg GABA/g of fresh weight. These results indicate genetic differences in GABA synthesis and accumulation due apparently to differences in protein content and its subsequent hydrolysis to amino acids by glutamate decarboxylase (Moongngarm & Saetung, 2010; Frias et al., 2005), such as glutamate, or amino L - glutamate (amino acid L-glutamate) which is the precursor of GABA (Watchararparpaiboon et al., 2012). When these GBR compounds were compared, with those of milled rice, they were 10 times greater for GABA, nearly 4 times greater for dietary fiber, vitamin E, niacin and lysine and 3 times greater for thiamine, pyridoxine and magnesium (Kayahara et al., 2000). Some studies are using GABA to develop GABA-rich foods (Kayahara et al., 2000). The number of pregerminated brown rice products is increasing on the Japanese food market because they contain higher amount of GABA and other nutritional components than the ordinary polished rice products. GABA from breads prepared from wheat flour, 30% of brown rice, 30% of germinated brown rice have 5.39, 8.60, and 32.05 mg/100 g, respectively (Cornejo et al., 2015). The content of GABA in sundried GBR has ranges from 12 mg/100 g DM in soaked grains to 67 mg/100 g DM in 34 ºC/96 h GBR. (Caceres et al., 2017). Moreover, it was reported that GBR could be used as a nutritive ingredient in many foods, including cookies, noodles, bread, rice-ball, tea, milk and breakfast cereals. The phytochemical profiles and antioxidant activity of free, soluble-conjugated, and bound fractions of brown rice and its processed products (textured rice, cooked rice and rice noodle) were studied. The total phenolic contents and antioxidant activities of free and soluble-conjugated fractions decreased after processing, the total-TPC (brown rice 132.29 mg GAE/100 g) of processed BR products ranged from 106.72 (rice noodle) to 129.65 mg GAE/100 g (textured rice). The total-FRAP (164.08 mg FE/100 g) of processed BR products ranged from 113.53 (rice noodle) to 140.35 mg FE/100 g (textured rice) (Er et al., 2017b).

2. Antioxidant activities

The antioxidant activity of brown rice and their products extracts were measured by DPPH-radical-scavenging and FRAP assay. The average quantity of the DPPH value in the rice flower extracts are shown in Table 2.

Table 2 The antioxidant activities by DPPH assay and FRAP of brown rice and their products (n=3)

Rice products	The DPPH assay was reported as IC_{50} (mg/mL)	FRAP (mM Fe (II) /g of fresh weight)
Brown rice	1.1±3.0	59.8 ±8.5
Germinated brown rice	3.1±4.4	33.7±7.7
Dry germinated brown rice	8.9±6.0	29.7±3.1
Germinated brown rice noodle	22.5±9.2	23.6±5.7
Dry germinated brown rice noodle	27±15	10. 0±5.0

The antioxidant activity of the extracts was analyzed using DPPH scavenging and FRAP assay. From Table 2, the brown rice shows the highest potential in scavenging DPPH radicals calculated in terms of IC_{50} (1.1±3.0 mg/mL). The possible mechanisms suggests that the radical-scavenging effects of rice might be due to the hydroxyl groups in the antioxidants of the extracts. Therefore, we suggest that the radical scavenging activity in the rice is from different antioxidants, such as gamma oryzanol, tocopherol and tocotrienol. Also, when the crops grow to sapling it can produce the secondary metabolites compounds, which include chlorophyll, oryzadione, 7-oxostigmasterol, and ergosterol peroxide, too (Lee et al., 2007). In contrast, the brown rice shows the highest FRAP value (59.8±8.5 mM Fe(II)/g of fresh weight). The FRAP activity in the phenolic extracts is related to the level of phenolic compounds. It is simple, fast and reproducible (Wong et al., 2006). It measures the ferric to ferrous reduction in presence of antioxidants, which are effective as secondary antioxidants because they reduce the redox potential. Moreover, the germination improves the organoleptic quality of brown rice because the enzymatic hydrolysis of the polymeric materials softens the rice kernels and often improves its flavor (Tian et al., 2004; Hunt et al., 2002). Therefore, it has been suggested that brown rice is utilized as a healthy ingredient in a variety of foods.

Conclusion

The results presented in this study reports on the correlation of antioxidant activity and phytochemical profile in brown rice and five brown rice samples (brown rice, germinated brown rice, dry germinated brown rice, germinated brown rice noodle and dry germinated brown rice noodle). The results show that brown rice and their products possess relatively strong antioxidant activity. The brown rice extract shows the highest of total phenolic, and GABA contents and antioxidant activities. There is also a high correlation between the total phenol content and antioxidant activities determined by DPPH ($R^2 = 0.781$) and FRAP ($R^2 = 0.541$). Past studies have reported the antioxidant activity and phytochemical compounds in brown rice and some of their products. The flavonoids were only detected in brown rice and germinated brown rice and presented data that had lower values than this study. The results suggest that phenolic compounds are the major contributors to the antioxidant activities of brown rice. In addition, germinated brown rice is a potential source of antioxidative, phytochemicals and it is a useful ingredient for nutraceutical, food industry, health products or functional food products. Moreover, further research is needed on isolation, purification, identification and quantification of each phenolic flavonoid and GABA compounds of brown rice and brown rice products.

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