Bioactive Compound Contents in Germinated Unpolished Purple Glutinous Rice from Kum Doi Saket and Kum Phayao Varieties

Somchai Jomduang

School of Agro-Industry, Faculty of Agro-Industry, Chiang Mai University 50100, Thailand

Corresponding author. E-mail: somchai.j@cmu.ac.th

ABSTRACT

This research studied the bioactive compounds in unpolished purple glutinous rice and germinated unpolished purple rice produced from two rice varieties: Kum Doi Saket and Kum Phayao. Unpolished purple glutinous rice grains were produced by grain dehusking without polishing. The quality of their physical, chemical and bioactive compounds was analyzed. Kum Doi Saket unpolished purple glutinous rice had a statistically significant ($p<0.05$) higher amount of almost all compositions than Kum Phayao, except ash and reducing sugar content. Germinated unpolished purple rice was produced by soaking in water at 40°C for 3 hrs, and then incubating at 35°C. The GABA content at 40 hrs of germination had the maximum amount in both varieties and tended to decrease after this time. Thus, 40 hrs of germination time was the optimum period for germinated rice from both rice varieties. When compared before and after the 40-hr germination period, gamma-oryzanol increased, but total anthocyanins and antioxidant activity decreased slightly. Comparing the two varieties of germinated unpolished purple glutinous rice grains, Kum Doi Saket had a statistically significant ($p<0.05$) higher amount of bioactive compounds and antioxidant activity than Kum Phayao. Germinated unpolished purple glutinous rice provided from Kum Doi Saket had 16.31±0.34 mg/100g of GABA, 30.48±1.61mg/100g of gamma-oryzanol, 70.10±0.45 mg/100g of total anthocyanins and 83.10±0.95% of antioxidant activity. This study has shown that the glutinous purple rice variety Kum Doi Saket had the highest potential for future development as a health food.

Keywords: Germinated rice, Purple rice, Gamma-aminobutyric acid, Gamma-oryzanol, Anthocyanins.

INTRODUCTION

Unpolished rice grain has a greater amount of certain nutrients, such as fiber, minerals, vitamin B1, vitamin B2 and vitamin E, than does the normal polished grain. In addition, unpolished rice grain contains many bioactive compounds, such as anthocyanins and gamma-oryzanol (Kayahara, 2001). Recently, it was reported

that a high amount of GABA (gamma-aminobutyric acid) was produced during rice germination. GABA is an amino acid that is good for human health in several ways, such as acting as a neurotransmitter in the central nervous system, which can prevent the increasing of blood pressure (Ohmori et al., 1987), relieving insomnia and depression symptoms (Kayahara and Tsukahara, 2000; Hayakawa et al., 2004; Sunte et al., 2007), improving memory and learning ability in mice (Miura et al., 2006), inhibiting leukemia cell proliferation and stimulating cancer cell apoptosis (Komatsuzaki et al., 2007). Currently, market trends show increasing consumption of unpolished rice.

In Thailand, some rice varieties have a dark purple seed coat; these are called "purple rice", "black rice" or "color rice". Most of the dark-purple color pigments are anthocyanins. This type of rice has higher gamma-oryzanol levels than normal polished rice (Puntipa et al., 2004). This compound can prevent the increasing of blood pressure, decrease the risk for diabetes (Kong et al., 2003) and decrease cholesterol and triglyceride levels in the blood (Sugano and Tsuji, 1997; Gerhardt and Gallo, 1998).

Northern Thailand has two important glutinous purple rice varieties: Kum Doi Saket and Kum Phayao. Both rice varieties have been reported to have a high content of some bioactive components and antioxidant activities (Puntipa et al., 2009 Utoomporn, 2011). Thus, this research aimed to study the quantities of bioactive compounds in unpolished purple glutinous rice and germinated unpolished purple glutinous rice produced from each rice cultivar. The optimal period for rice germination in which the highest amount of GABA could be obtained was also studied. This knowledge could be commercially applied in the product development of germinated unpolished purple glutinous rice.

MATERIALS AND METHODS

Determination of physicochemical qualities of unpolished purple rice

Paddies containing Kum Doi Saket and Kum Phayao varieties were processed by paddy dehusker (Satake, Japan) to obtain unpolished purple glutinous rice. Proximate chemical compositions such as moisture content, protein, fat, fiber, ash and carbohydrate were determined (AOAC, 2000). Amylose content, reducing sugar and germination rate were also evaluated using the method described by Knutson (1986) and ISTA (1988) respectively. Other bioactive compounds, such as GABA and gamma-oryzanol, were analyzed using HPLC with Ultra C18, 5 µm 250 x 4.6 mm column and 1.0 ml/min flow of gradient mobile (Xu and Godber, 1999; Timothy et al., 2010). Total anthocyanins and antioxidant activity {2,2-diphenyl-1-pycryl hydrazyl (DPPH) scavenging activity} were also determined using a spectrophotometer (Ranganna, 1986). All data were determined in triplicate, and statistical analyses were compared using a pair-sample T-test ($p \leq 0.05$).

Determination of the optimal germination time of unpolished purple rice

Unpolished purple glutinous rice from each rice variety was soaked at 40°C for 3 hrs and incubated at 35°C (Voranuch, 2009). After 24 hrs of incubation,

the germinated rice was collected every 8 hrs and subsequently dried until the moisture content was around 13% (w/w), using a hot air oven at 50°C. The dried germinated rice grains were milled using a hammer mill, and GABA content was determined following experiment 1. The optimal germination time was selected as the incubation time that had the highest GABA content. The quantity of gamma-oryzanol, total anthocyanins and antioxidant activity were also determined. All data were determined in triplicate and statistical analyses were compared using a pair-sample T-test ($p \leq 0.05$).

RESULTS

Physicochemical qualities of unpolished purple rice

Analysis of the physicochemical qualities of Kum Doi Saket and Kum Phayao cultivars demonstrated some slight differences, such as 90.33±1.52 and 81±1.00% of grains for germination rates, 10.78±0.02 and 11.42±0.07% of moisture, 8.56±0.09 and 6.89±0.06% of protein, 2.57±0.12 and 2.12±0.10% of fat, 1.86±0.18 and 1.60±0.12% of crude fiber and 74.83±0.07 and 76.50±0.19% of carbohydrate, respectively (Table 1). In addition, ash content, reducing sugar and amylose contents of Kum Doi Saket unpolished rice grain were 1.40±0.07, 0.22±0.01 and 1.49±0.01%, respectively, which were similar to Kum Phayao unpolished rice. The unpolished rice of Kum Doi Saket and Kum Phayao contained bioactive compound content of 0.84±0.02 and 0.65±0.02 mg/100 g of GABA, 22.55±1.81 and 18.51±1.73 mg/100 g of gamma-oryzanol, 78.48±10.2 and 117.19±2.30 mg/100 g of total anthocyanins and 90.97±0.52 and 75.83±0.96% of antioxidant activities (DPPH assay), respectively (Table 1). Kum Doi Saket's bioactive compounds were significantly higher than those in Kum Phayao ($p \leq 0.05$).

The optimal germination time of unpolished purple rice

When unpolished rice grains from Kum Doi Saket and Kum Phayao were incubated for germination at 35°C for 24 hrs, the GABA contents of both rice varieties tended to increase. At 40 hrs of incubation time, they had the maximum amount of GABA and then tended to decrease after that (Figure 1). Thus, both rice varieties had the same suitable incubation time at 40 hrs, which yielded 16.31±0.34 and 12.83±0.13 mg/100 g dry weight of GABA, respectively. At that time, both grains changed their semblances with the presence of small tips and roots (Figure 2). Comparing GABA content in non-germinated and germinated purple rice of both Kum Doi Saket and Kum Phayao indicated that after the 40-hr germination time, GABA content increased by about 19.2 and 21.4 times, respectively (Figure 3).

When the bioactive compounds and antioxidant activity of germinated unpolished purple glutinous rice before and after incubation for 40 hrs were compared, both rice varieties after germination had a higher amount of gamma-oryzanol, but a slightly smaller amount of total anthocyanins and antioxidant activity (Table 2). When the germinated rice was compared between both varieties, Kum Doi Saket had a significantly higher amount of bioactive compounds than Kum Phayao ($p \leq 0.05$), including GABA content (16.31±0.34 mg/100 g dry weight),

gamma-oryzanol content (30.48±1.61 mg/100 g dry weight), total anthocyanins content (70.10±0.45 mg/100 g dry weight) and antioxidant activity (83.10±0.95 mg/100 g dry weight).

Table 1. Comparison of quantitative components from two purple glutinous rice varieties (Kum Doi Saket and Kum Phayao).

Qualities	Unpolished purple glutinous rice[1/]	
	Kum Doi Saket	Kum Phayao
Physical composition		
Germination rate (% of grains)	90.33 [a] ± 1.52	81 [b] ± 1.00
Chemical compositions		
Moisture (%)	10.78 [b] ± 0.02	11.42 [a] ± 0.07
Protein (%)	8.56 [a] ± 0.09	6.89 [b] ± 0.06
Fat (%)	2.57 [a] ± 0.12	2.12 [b] ± 0.16
Ashns (%)	1.40 ± 0.07	1.47 ± 0.10
Crude fiber (%)	1.86 [a] ± 0.18	1.60 [b] ± 0.12
Carbohydrate (%)	74.83 [a] ± 0.07	76.50 [b] ± 0.19
Reducing sugarns (%)	0.22 ± 0.01	0.26 ± 0.02
Amylose (%)	1.86 [a] ± 0.18	1.60 [b] ± 0.12
Bioactive compounds		
GABA (mg/100g DW)	0.85 [a] ± 0.01	0.60 [b] ± 0.02
Gamma-oryzanol (mg/100g DW)	22.55 [a] ± 1.81	18.51 [b] ± 1.73
Total anthocyanin (mg/100g DW)	117.19 [a] ± 2.30	78.48 [b] ± 1.02
Antioxidant activity		
DPPH scavenging activity (%)	90.97 [a] ± 0.52	75.83 [b] ± 0.96

Note: [1/]For the comparison in each row, the different letters indicated the statistically significant difference ($p<0.05$). ns not a significant difference.

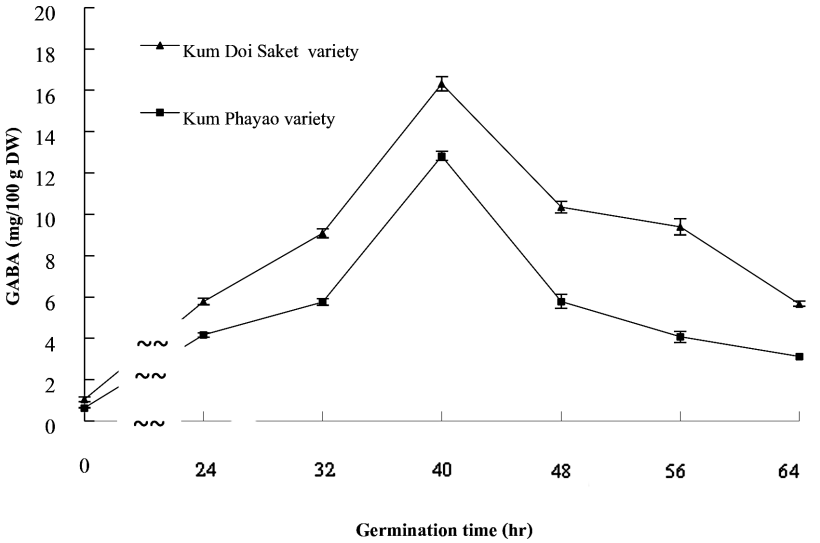

Figure 1. GABA content of unpolished purple glutinous rice during germination period.

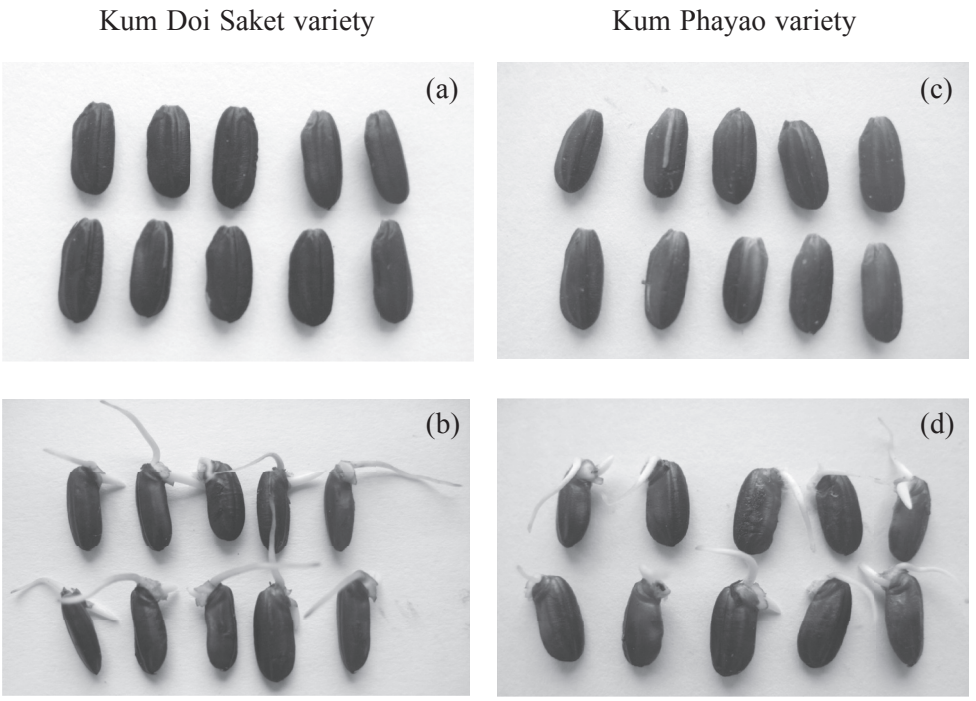

Figure 2. Unpolished purple rice [(a) and (c)] and germinated unpolished purple rice[(b) and (d)] grains from two varieties.

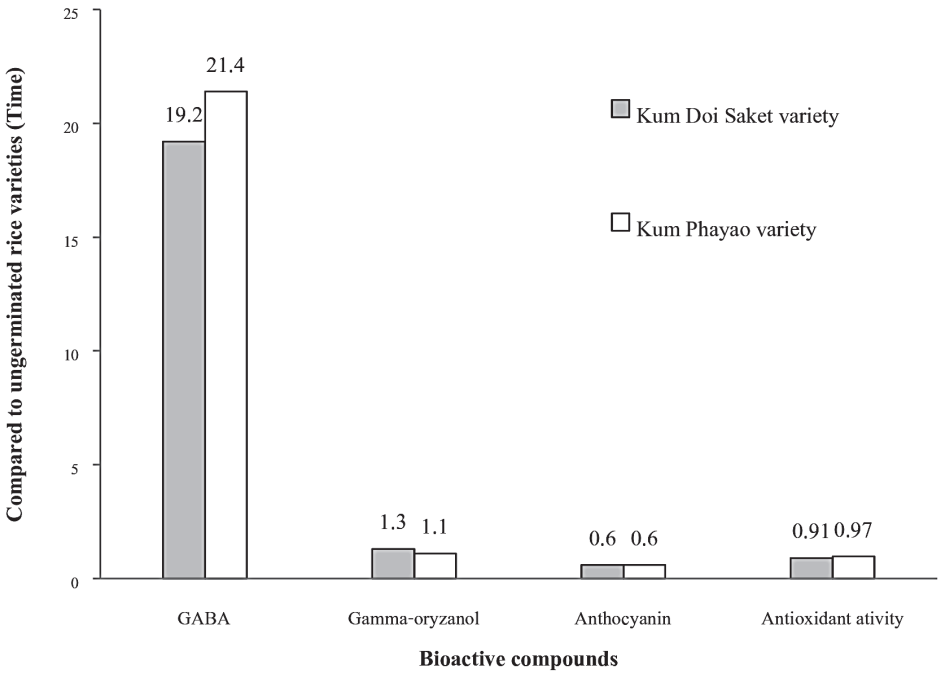

Figure 3. Changes of bioactive compound contents in each variety of germinated unpolished purple rice compared with ungerminated rice varieties.

Table 2. Bioactive compound contents of purple rice (Kum Doi Saket and Kum Phayao) at 40-hr germination time.

Bioactive compound contents	Germinated unpolished purple glutinous rice[1/]	
	Kum Doi Saket	Kum Phayao
GABA (mg/100g db)	16.31 a ± 0.34	12.83 b ± 0.13
Gamma-oryzanol (mg/100g db)	30.48 a ± 1.64	20.20 b ± 1.61
Total anthocyanin (mg/100g db)	70.10 a ± 0.45	45.26 b ± 0.38
DPPH scavenging activity (%)	83.10 a ± 0.95	74.28 b ± 0.78

Note: [1/]For the comparison in each row, the different letters indicated the significant statistical difference ($p<0.05$). db = dry weight basis.

DISCUSSIONS

Physicochemical qualities of unpolished purple rice

Both Kum Doi Saket and Kum Phayao rice varieties can be classified as glutinous rice cultivars due to their very low amylose content. Some of the physicochemical qualities varied between the varieties. These results showed some slight differences from previous studies. Utoomporn (2011) reported that Kum Doi Saket had 16.72±0.02% moisture, 8.90±0.12% protein content, 2.32±0.04% fat, 1.41±0.03% ash, 70.65±0.05% carbohydrate and 0.24±0.02% reducing sugar. Dumnern et al. (2000) reported the nutritional values of 27 purple glutinous rice varieties, which were 8.58-11.75% of protein, 1.37-3.84% of fat, 1.63-2.13% of ash and 81.04-85.68% of carbohydrates. Germination rates of grains were important for GABA production during germination. Kum Doi Saket, which had 90.33±1.52% germination rates, had a higher potential GABA content after germination than did Kum Phayao.

Regarding bioactive compound content, Kum Doi Saket's bioactive compounds were significantly higher than those of Kum Phayao ($p<0.05$). In addition, anthocyanins and gamma-oryzanol contents in Kum Doi Saket from this study were higher than those observed in the report of Puntipa et al. (2009) and Utoomporn (2011). It was reported that not only the difference of rice varieties but also the difference of these chemical compositions and bioactive compounds depended on various parameters, such as rice cropping, fertilization, handle harvesting and storage time of grains (Moongngarm and Saetung, 2010).

The optimal germination time of unpolished purple rice

Comparison of GABA content in non-germinated and germinated purple rice for both Kum Doi Saket and Kum Phayao showed that GABA contents increased by about 19.2 and 21.4 times, respectively, when germinated for 40 hrs. These results were similar to some reports from other studies. Voranuch (2009) reported that germinated rice from Chai Nat and Jasmine rice cultivars, incubated at 35°C for 20 hrs, showed that GABA content increased by about 16.8 and 19.6 times, respectively. Anawachkul and Jiamyangyuen (2009) reported that the GABA content in unpolished Munpoo rice, soaked in water for 24 hrs and incubated in the dark for 48 hrs, was about 8.4 times higher than in ungerminated rice.

In addition, Ohtsubo et al. (2005) reported that the optimal incubation time of the Koshihikari rice cultivar was 96 hrs and the GABA content was 24.7 times higher than the original amount. From this study, optimal germination time was slightly shorter than that recorded in other Thai rice studies, but it could result in a higher amount of GABA content.

After germination, both rice varieties tended to show an increased amount of gamma-oryzanol, but a decreased amount of total anthocyanins and antioxidant activity. Jirapong and Jiamyangyen (2012) also reported the decreasing of anthocyanins content and radical scavenging after germination. On the other hand, Moongngarm and Saetung (2010) reported a 1.3 times increase in gamma-oryzanol after germination. The decreasing of anthocyanins may be caused by their solubility levels in water during soaking. This study has shown that Kum Doi Saket had a significantly higher amount of bioactive compounds than Kum Phayao ($p \leq 0.05$), including GABA contents (16.31±0.34 mg/100 g dry weight), gamma-oryzanol contents (30.48±1.61 mg/100 g dry weight), total anthocyanins contents (70.10±0.45 mg/100 g dry weight) and antioxidant activity (83.10±0.95 mg/100 g dry weight).

CONCLUSION

Kum Doi Saket rice grain had a higher germination rate, GABA (gamma-aminobutyric acid), gamma-oryzanol and total anthocyanins and antioxidant activity than Kum Phayao. Incubation of unpolished rice grains at for 40 hrs at 35°C was the optimal germination time for both rice cultivars. After 40 hrs germination, Kum Doi Saket had more bioactive compounds than Kum Phayao, especially GABA and antioxidant activity. Therefore, of the two glutinous purple rice varieties studied here, Kum Doi Saket has the highest potential for future development as a health food.

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