Physiochemical, Antioxidant Activities and Anthocyanin of Riceberry Rice from Different Locations in Thailand

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Abstract

The objective of this study was to investigate the physiochemical qualities, antioxidant activities and anthocyanin content of Riceberry rice for the selected locations in Thailand. The study on the Riceberry rice was conducted from the five locations: Chiang Mai, Lampang, Phetchabun, Sing Buri, and Surin provinces; they were evaluated on the qualities such as length, width and color values (L*, a*, and b*), proximate composition, total phenolic content (TPC), DPPH radical scavenging activity assay, ABTS radical scavenging activity assay and anthocyanin content. The results indicated that the difference in areas were significantly different of L* a* and b* values (p<0.05). The percentage of moisture content was in the range of 9.69 to 11.59. The fat contents were in the range of 2.46% to 3.35%. The percentage of protein, ash, fiber and carbohydrate contents was in the scope of 7.84–9.25, 9.69–11.59, 1.82–2.40 and 73.45–76.50, respectively. The quantitative composition of total phenolic compound, % scavenging ability of DPPH, % scavenging ability of ABTS, anthocyanin content of Riceberry rice in different areas all had a significant difference (p<0.05). As for total phenolic compound and total anthocyanin the content was between 179.16–327.61 mgGAE/100g and 24.69–272.76 mg/100g of rice, respectively. The percentage scavenging ability of DPPH and percentage scavenging ability of ABTS was in the range of 39.25 to 71.54 and 27.29 to 43.03, respectively.

Keywords: Riceberry rice, Physical, Chemical, Antioxidant

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1. Introduction

Riceberry rice, a new valuable rice variety of Thailand, is a popular consumption significantly with the locals. Riceberry rice is a deep purple grain; (*Oryza Sativa*), a cross-breed strain from the Khao Hom Nin Rice variety which is well known containing high antioxidant properties and Khao Hom Mali 105 which is well known as a fragrant rice (Kongkachuichai *et al.,* 2013). It contains poly-phenols compounds, anthocyanin, vitamin E and Gamma-Oryzanol, acting in the antioxidant ability. Anthocyanin is the main active chemical component which is found in Riceberry rice. The key element in anthocyanin is cyadinin and peonidin which is found in a pigment substance on the surface layer of rice grain or rice bran (Leardkamolkarn *et al*., 2011; Sirichokworrakit *et al*., 2015). There is a growing interest in rice consumption due to the frequency of consuming nutrition that is associated with reducing risk of diseases, such as cancer, cardiovascular heart attack, diabetes (Prangthip *et al.*, 2013), and high blood pressure (Leardkamolkarn *et al*., 2011; Sirichokworrakit *et al*., 2015).

Riceberry rice requires special attention. Rice must be grown in the process of organic farming in cold weather to create a production of rice seed color. The selection of planting area is important. A good area of planting Riceberry rice should have a relatively high fertility of the natural soil. It contains nutrients necessary for the growth of rice adequately. There is a source of water for cultivation. Areas should not be a space with the use of chemicals in large doses for long periods, otherwise the chemical contamination will be very high and hazardous for eating. The cultivation from that area uses a lot of agricultural chemicals. The Riceberry Rice growing area has a significant effect on rice quality. Previous research studies have found that the growing area has a different effect on the quality of rice. Somthawil and Sriwattana (2016) reported Sensory profiles from a descriptive analysis, physical properties and consumer acceptance were used to compare qualities among purple rice growing in six different locations of cooked purple rice. Jing and Yuan (2012) showed the effects of different altitude on rice yield and quality in China. Zhu *et al.* (2010) found that there are effects of altitude on cooking and eating the rice. Su *et al.* (2008) reported that the altitudes has an effect on the amylose content. The objective of this research was to investigate the physicochemical and antioxidant activity of Riceberry rice from five different growing areas in Thailand. In this study there was a supplied theoretical database to select the location in the promotion of planting quality Riceberry rice as well as getting technical support in the functional product applications for further usage.

2. Materials and Methods

2.1 Rice samples

Riceberry rice, which was milled rice, was collected from the different locations of Thailand. The locations of five Riceberry rice were the Saraphi district, Chiang Mai province (in the northern region of Thailand), Mueang Pan district, Lampang province (in the northern region of Thailand), Si Thep district, Phetchabun province (in the central region of Thailand), Mueang district, Sing Buri province (in the central region of Thailand), and Mueang district, Surin province (in the northeastern region of Thailand). All samples of Riceberry rice were harvested in 2016. These samples were stored at 4°C in a vacuum pack for analysis.

2.2 Physical properties of Riceberry rice

2.2.1 Length and Width

Length and width of rice seed were determined using method of Kanchana *et al*. (2012). Ten grains were arranged horizontally (for length) and vertically (for width) along the X- axis of the graph sheet and the total number of units occupied were counted. Scale one unit equal to 1 cm. Total length and width was averaged and the length and the width of one grain was found out.

2.2.2 Color

A colorimeter (Color Global, Mini-scan EZ, Hunter Lab, America) were used to measure the color of rice samples and expressed as L*, a*, and b* values. It was the definitions, L*, represents lightness (L* = 100 (lightness), L* = 0 (darkness), a* is the red/green coordinate (+a* redness, -a* greenness), and b* is the yellow/blue coordinate (+b* yellowness, and –b* blueness).

2.3 Proximate composition

Moisture, protein, fat, fiber and ash contents were determined in triplicates according to method described by AOAC (2000). The carbohydrate content (estimated total carbohydrate content) were determined by the difference between 100 and the sum of the percentages of moisture, protein, fat, fiber and ash.

2.4 Total phenolic content (TPC)

Rice sample was ground by an electric blender, and then extracted in 85% of aqueous methanol solution (ratio 1:10) under mild agitation using magnetic stirrer for 30 min. The extract solution was centrifuged at 5000 rpm for 10 min, and filtered through a 0.45 μm nylon filter. The supernatant was separated from the residue and stored at 4ºC until analysis. The rice extract was used to further determine TPC and free radical scavenging activity.

Total phenolic content (TPC) in each of rice extracts were determined by colorimetric method (Sompong *et al*., 2011). Briefly, 20 μL of extract were reacted with 100 μL of 0.2 M

Folin-Ciocalteu’s reagent for 1 min. Then, 80 µL of 7.5% (w/v) Na_2 CO_3 were added into the reaction mixture. After incubation at room temperature for 30 min, the absorbance of extract were measured using spectrophotometer at 765 nm.

2.5 DPPH radical scavenging activity assay

DPPH radical-scavenging ability of rice extracts were evaluated according to the procedure of Brand-Williams (Sompong *et al*., 2011). The reaction mixture was contained 1.5 mL of DPPH working solution (4.73 mg of DPPH in 100 mL ethanol solution) and 300 µL of rice extract. The mixture was shaken and incubated for 40 min in the dark at room temperature. The absorbance was read at 515 nm using a spectrophotometer.

2.6 ABTS radical scavenging activity assay

ABTS radical scavenging activity of extract was measured. The ABTS radical scavenging of extracts was analyzed by modified ABTS cation decolorization assay as described by Re *et al*. (1999) and Wojdylo *et al*. (2007). The working $ABTS^+$ solution was freshly prepared by mixing of 7 mM ABTS and 2.4 mM potassium persulfate in equal quantities and allowed to react for 12 h at room temperature in the dark condition. The mixed solution was diluted with ethanol to obtain an absorbance of 0.95±0.01 units at 734 nm. The extracted sample (70 µL) was reacted with 630 µl of the mixed solution. After 30 min of incubation at room temperature, the absorbance was spectrophotometrically measured at 734 nm.

2.7 Anthocyanin profile

Three grams of the ground materials was extracted by mixing with 30 mL of methanol acidified with 1% HCl (v/v) and shaking on rotatory shaker at 200 rpm for 1 h. The extracts were centrifuged at 6000g and 5°C for 20 min. The supernatant was concentrated and filtered through 0.45 µL syringe filter before being injected to HPLC.

Anthocyanins in the extracts were separated and quantified with a High performance liquid chromatography (HPLC, Agilent Technologies, Santa Clara, CA, USA) as in Rattanachaisit and Kongkiattikajorn, 2015; Laokuldilok *et al*, 2011. A C18 rapid resolution column was employed for separation. The mobile phase was the mixture of water, methanol and formic acid (75:20:5 v/v) with isocratic elution at 0.5 mL/min flow rate. The separated anthocyanins were detected and measured at 530 nm, and the sample loop was 25 µL was quantified by a reverse-phase HPLC method at 530 nm using a standard curve generated by cyanidin 3- glucoside and peonidin 3-glucoside.

2.8 Statistical data analysis

The data obtained were analyzed statistically to find out the best source of Riceberry rice using Complete Randomized Design (CRD). All data analyzed was carried out in triplicate and reported as mean ± standard deviation of mean (SD). The Duncan’s Multiple Range test

(DMRT) was used to analyze mean separation using the SPSS 17.0 (SPSS Inc., IBM Corp., IL, USA) program with the significant level determined at 95% confidence limit (P<0.05).

3. Results and Discussion

3.1 Physical properties of Riceberry rice in different locations

The results of the study, L* a* and b* values of Riceberry rice from the difference of areas in Thailand are reported in Table 1. The results showed that Riceberry rice growing in Sing Buri possess greatest values of L* a* and b* (p<0.05) of Riceberry rice. Riceberry rice had a deep purple color. According to the findings by Jittorntrum *et al*. (2009), rice bran extracts of dark violet grain are called Riceberry because the color characteristics of Riceberry rice look like one of the berry fruit family (Sirichokworrakit *et al*, 2015). For the size of the rice grain, their width did not have a significant difference (p>0.05). The width of Riceberry rice seeds were in the range of 1.98 to 2.01 cm. While, the length of Riceberry rice seeds had a significant difference (p<0.05). The length of rice from Chiang Mai and Surin were higher than the length of rice from others.

Table 1 Color as L* a* b* value, length and width of Riceberry rice in different locations

Location	L*	a*	b*	Length(cm)	Width (cm)^{ns}
Chiang Mai	19.68±0.11^{b}	3.57±0.06^{b}	2.48±0.15^{b}	6.63±0.33^{a}	2.00±0.12
Lampang	19.63±0.06^{b}	2.34±0.11^{c}	2.22±0.39^{b}	6.18±0.63^{b}	2.01±0.12
Phetchabun	18.10+0.08^{c}	3.32±0.20^{b}	2.30±0.24^{b}	6.43±0.43^{a,b}	2.01±0.07
Sing Buri	20.56±0.29^{a}	5.74±0.63^{a}	4.47±0.50^{a}	6.25±0.26^{b}	1.98±0.11
Surin	18.96±0.07^{c}	3.67±0.33^{b}	2.31±0.09^{b}	6.72±0.29^{a}	2.00±0.18

Note: $^{a\text{-}c}$ Different letters in the same column indicate significant difference between means (DMRT, p<0.05).
^{ns} letters indicate non-significant difference between means in the same column (DMRT, p>0.05).

3.2 Proximate compositions of Riceberry rice in different locations

The proximate compositions of the Riceberry rice from the different areas in Thailand are shown in Table 2. The results showed that there were significant difference in all the compositions (p<0.05). The percentage of moisture content of Riceberry rice from Sing Buri and Phetchabun province were evidently lower than that of Chiang Mai, Lampang and Surin province. The fat content of Riceberry rice from Surin province revealed the highest value while, protein content of Riceberry rice from Lampang and Surin province were not significant difference. The percentage of Riceberry rice ash from Phetchabun province possess the highest value. The percentage of fiber and carbohydrate of riceberry rice from Sing Buri and

Phetchabun province were higher than that of Chiang Mai, Lampang and Surin province, respectively. In agreement with the study by Chuaykarn *et al*. (2013), they also found that the moisture, protein, fat, ash, crude, fiber and carbohydrate contents of Riceberry rice were in similar result. In Addition, Jing and Yuan (2012) also found that the nutritional quality of rice depend on the planting at the altitude.

Table 2 Proximate composition of Riceberry rice in different location

Source (Provinces)	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Chiang Mai	11.20±0.48b	2.75±0.03b,c	8.73±0.10b	1.30±0.07b,c	2.13±0.11b	73.70±0.35b
Lampang	11.59±0.11b	2.63±0.10b,c	9.22±0.08a	1.08±0.12d	1.82±0.11c	73.60±0.26b
Phetchabun	9.69±1.26a	2.59±0.25b	8.13±0.70b	1.63±0.04a	2.40±0.10a	75.56±1.70a
Sing Buri	9.71±0.12a	2.46±0.04c	7.84±0.07c	1.19±0.02c,d	2.40±0.20a	76.50±0.31a
Surin	10.50±0.02a,b	3.35±0.01a	9.25±0.10a	1.38±0.01b	2.07±0.07a,b	73.45±0.13b

Note: $^{a-c}$ Different letters in the same column indicate significant difference between means (DMRT, p<0.05).

3.3 Total phenolic compound and total anthocyanin content of Riceberry rice in different locations

The results of total phenolic compound and total anthocyanin content of Riceberry rice in different locations were reported in Figure 1. Total phenolic compound of Riceberry rice from different locations indicated a significant difference (p<0.05). Total phenolic compound of samples were found in the range of 179.16 to 327.61 mgGAE/100g. The Riceberry rice of Lampang and Phetchabun provinces had higher total phenolic contents than other samples. These phytochemical compounds usually accumulated in pericarp or bran of rice kernels (Mira *et al*., 2009; Laokuldilok *et al.*, 2011). It exhibited purple or black rice which is more plentiful in anthocyanin and other phenolic compounds compared to that of white rice (Zhang *et al*., 2006). For the reason that Sompong *et al*. (2011) demonstrated anthocyanin there was some main compounds of total phenolic content in Riceberry rice. This study found that total anthocyanin content of Riceberry rice from different locations indicated a significant difference (p<0.05). Total anthocyanin content of Riceberry rice from Lampang provinces was the highest value. There is a hot spring in Mueang Pan district, Lampang province. The anthocyanin compositions seem to be related to the location (Cheng, 2014) close to hot spring which possess high temperature and chemical composition. Moreover, Kushwaha (2016) was observed that the anthocyanin content of black rice increased significantly with increase in altitude of its plantation site. In addition, the attitude of Lampang is 500 meter above mean sea level.

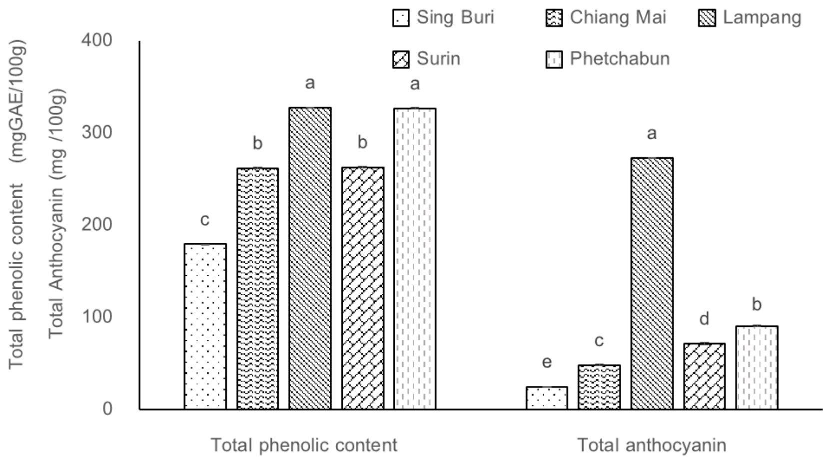

Figure 1 Total phenolic compound and total anthocyanin content of Riceberry rice in different locations

Note: [a-e] Different letters indicate significant difference treatment (DMRT, p<0.05)

3.4 Cyanidin and peonidin content of Riceberry rice in different locations

The result of cyanidin and peonidin content of Riceberry rice in different locations is shown in Figure 2. Anthocyanin in Riceberry rice contain 2 main compounds of anthocyanin; cyanidin 3-glucoside (C3G) and peonidin 3-glucoside (P3G) as reported by Jittorntrum *et al*. (2009). This study found that cyanidin and peonidin of Riceberry rice from different locations showed a significant difference (p<0.05). The cyanidin and peonidin content of Riceberry rice from Lampang province revealed the highest value of cyanidin and peonidin which was 272.76 and 56.80mg/100g DW, respectively. Moreover, Jittorntrum *et al*. (2009), reported that Riceberry rice have contained cyanidin and peonidin in the amount of 150.81 and 66.76mg/100g, respectively. These compounds generally found pigment of fruits, vegetables, and colored rice having important roles in reducing the risk of cancer (Wang and Stoner, 2008).

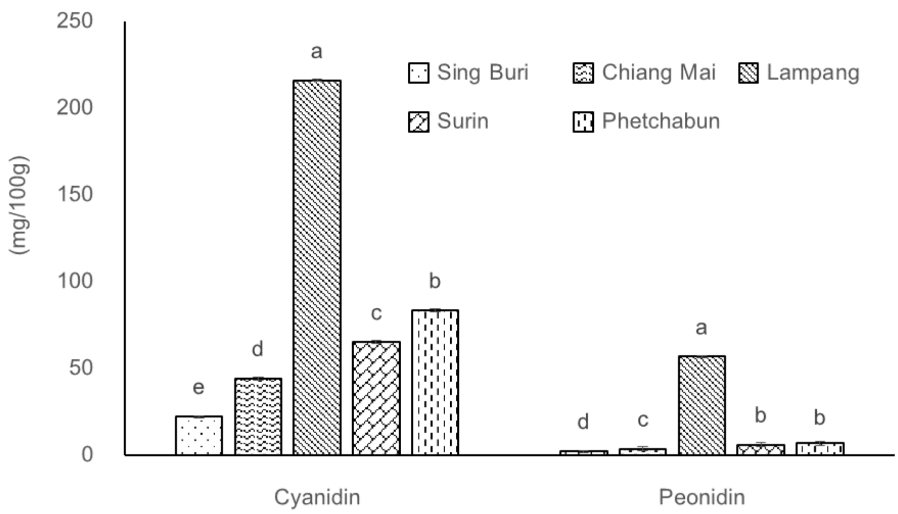

Figure 2 Cyanidin and Peonidin content of Riceberry rice in different locations

Note: [a-e] Different letters indicate significant difference treatment (DMRT, p<0.05)

3.5 Antioxidant activity of Riceberry rice in different locations.

The quantitative composition of % scavenging ability of DPPH and % scavenging ability of ABTS different locations were summarized in Figure 3. Obtained results indicated a significant difference ($p<0.05$). It was found that Riceberry rice from Phetchabun province has the highest % scavenging ability of DPPH, followed by Lampang province with 71.54 and 64.07 respectively. While, samples of Sing Buri and Lampang province did not have a significant difference of high content of % scavenging ability of ABTS which was 43.41 and 43.03, respectively. The ABTS and DPPH assay was based on an electron transfer and involves reduction of a colored oxidant (Floegel *et al*., 2011). Anthocyanin is able to capture free radicals by donation of phenolic hydrogen atoms, this is the reason for its antioxidant capacity (Castañeda-Ovando *et al*., 2009). Shao *et al*. (2018) also revealed correlation analyses confirmed that phenolic significantly contributed to the antioxidant activity of color rice.

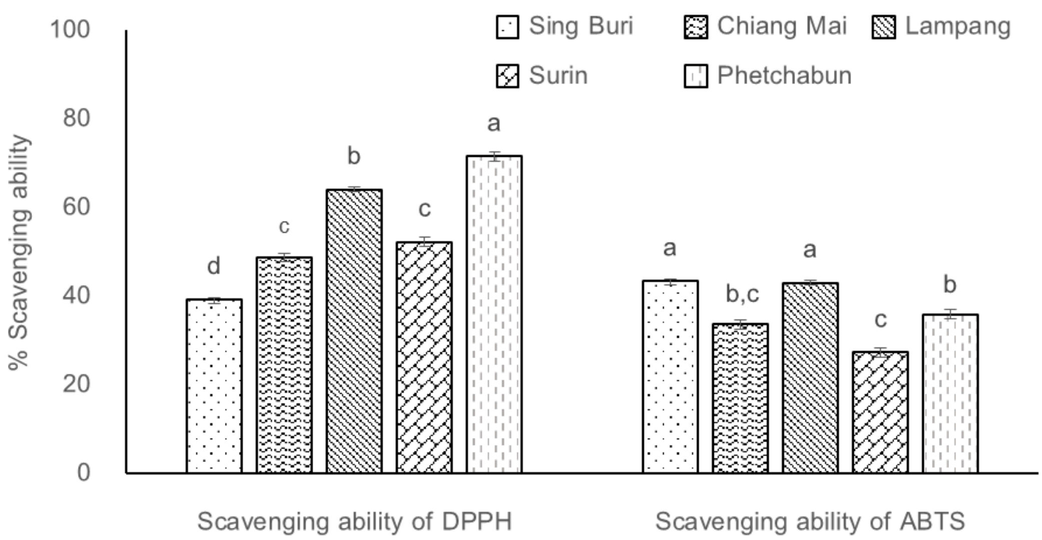

Figure 3 Scavenging ability of DPPH and scavenging ability of ABTS of Riceberry rice in different locations

Note: [a-c] Different letters indicate significant difference treatment (DMRT, $p<0.05$)

4. Conclusion

The findings of the present study based on the five locations of growing area of Riceberry rice in Thailand documented the physical properties, proximate compositions, antioxidant properties and anthocyanin content. It can be concluded that the present work clarifies the effects of Lampang Riceberry cultivation on antioxidant activities and anthocyanin profiles, thus providing guidance for production of high-quality Riceberry rice in different regions. Future research is needed for sensory evaluation on different locations of Riceberry rice.

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