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Contributed Paper

Stability of Anthocyanin Content and Antioxidant Capacity Among Local Thai Purple Rice Genotypes in Different Storage Conditions

Supaporn Yamuangmorn [a], Bernard Dell [b], Benjavan Rerkasem [c] and Chanakan Prom-u-thai* [a,d]

[a] Agronomy Division, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

[b] Office of the Deputy Vice Chancellor (Research), Murdoch University, Perth 6150, Australia.

[c] Plant Genetic Resources and Nutrition Laboratory, Chiang Mai University, Chiang Mai 50200, Thailand.

[d] Lanna Rice Research Center, Chiang Mai University, Chiang Mai 50200, Thailand.

* Author for correspondence; e-mail: chanakan15@hotmail.com; chanakan.p@cmu.ac.th

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ABSTRACT

This study determined whether storage conditions over six months affected grain anthocyanin content and antioxidant capacity of four purple rice genotypes with different initial anthocyanin content. Two forms of grain, paddy and brown rice were stored at three temperatures -25 °C, room temperature (25-30 °C) and 60 °C with three replications. In the two high-anthocyanin genotypes, the anthocyanin continued to decrease until the last month of storage, while the low-anthocyanin genotypes were not affected by storage time. Storage at 60 °C decreased the average anthocyanin content by 42%, compared to the 30% loss for grain stored at either -25 °C or room temperature. Brown rice retained more of its anthocyanin than paddy rice in the high anthocyanin genotypes. Antioxidant capacity substantially declined during storage in all genotypes and the capacity was higher in brown than paddy rice. There was a significant correlation between anthocyanin content and antioxidant capacity in the two high-anthocyanin genotypes, but not in the low-anthocyanin genotypes, indicating the possibility of different anti-oxidative key compounds between the low- and high-anthocyanin genotypes. These results suggest that the anthocyanin content and antioxidant capacity in purple rice genotypes would be most stable when stored at low temperature as brown rice.

Keywords: pigmented rice, colored rice, storage condition, anthocyanin, antioxidant stability

1. INTRODUCTION

Pigmented rice is a special type of the rice species, which has both non-glutinous and glutinous rice and consume as unpolished rice with high level of nutrients [1]. Purple rice is also known as black rice which is a

major type of pigmented rice and mainly cultivated in Asia such as China, India and Thailand [2]. Purple rice, as a rich source of biological compounds, has become a popular health food product. The purple pericarp is

reported to contain suites of compounds with anti-oxidative properties, including flavones, phenols, sterols, tocopherols, γ -oryzanols, amino acids and essential oils [3]. Anthocyanin in purple rice has been reported to have health benefits, including reducing risks of cardiovascular disease, decreasing atherosclerotic plaque formation and increasing antioxidant status [4-6]. Hence, purple rice is being used as natural color in the food processing industry, as well as in dietary supplements, cosmetics and pharmaceutical products.

After harvest, rice is stored for later consumption and commercial trading purposes, including product development. The stability of the beneficial compounds in purple rice during storage remains a concern. Grain moisture content and storage temperature have been reported to affect the chemical, physical and functional qualities of purple rice [7]. High storage temperatures degraded the phenolic acids in brown and milled rice [8]. Furthermore, in purple rice, extracting anthocyanin at 100 °C accelerated its degradation compared with 80 or 90 °C [9]. Thus, storage temperature affects the stability of biological compounds in purple rice, but the association between anthocyanin content and antioxidant capacity during storage is unknown as well as the suitable form of rice to retain the beneficial compounds during storage. This study investigated the effect of three storage temperatures for six months on the anthocyanin content and antioxidant capacity of high- and low-anthocyanin rice genotypes. The effects of storage temperatures were evaluated for both paddy (un-husked grain) and brown (husk removed) rice. The research will help to identify protocols to maintain the stability of purple rice for the health benefit of rice consumers.

2. MATERIALS AND METHODS

2.1 Plant Culture

Four purple rice genotypes with low (Kum Wiengsa and Kum Doi Saket; range from 9 to 12 mg/ 100 g) and high (Kum 7677 and Kum Fang; range from 30 to 55 mg/ 100 g) anthocyanin content were used in this study. The selected genotypes were grown in the research field of the Agronomy Division, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Thailand in the wet season 2013 under the same field and management conditions to avoid an environmental effect on grain anthocyanin content. Rice seeds were soaked in water overnight, incubated moist until germinated and grown for 30 days in a seedbed. Individual seedlings were transplanted into hills at 25 × 25 cm spacing between plants; the plot size was 3 × 4 m. Nitrogen fertilizer was applied at 120 kg N ha⁻¹, half at maximum tillering and half at flowering. The field was kept flooded under 0.1 - 0.2 m of water until maturity. Rice seed was harvested at maturity and rapidly kept in the common woven polyethylene bags in cold room (18 °C) before being prepared and treated in the different storage conditions.

2.2 Sample Preparation

Rice grain of un-husked samples of each genotype was de-husked to produce brown rice with a laboratory husker (Model P-1 from Ngek Seng Huat Co. Ltd., Thailand) in three replications. The brown rice was analyzed for initial anthocyanin content. The remaining un-husked samples were divided into two parts, about 200 g each. The first part was de-husked as described above. The second part remained in the un-husked form as paddy rice. Subsamples of about 20 g of each genotype and form were stored in sealed plastic bags at three

temperature conditions (-25 °C, room temperature (25-30 °C) and 60 °C) in the dark for six months, February to July 2013. The experiment was arranged in factorial in CRD with three independent replications with independent factors of genotypes, storage time and temperature and grain form. The samples were subsampled three times at each replication for each month for anthocyanin and anti-oxidative capacity measurements. The un-husked samples were de-husked with a husker machine to yield brown rice before analysis.

2.3 Chemical Analysis

2.3.1 Anthocyanin content

Anthocyanin content was determined by the modified pH-differential method of Abdel-Aal and Hucl [10]. Whole grain (2.5 g) was transferred into a test tube containing 24 mL deionized water in a 50 °C water bath with shaking every 5 min for 30 min, and then the liquid was filtered through Whatman No. 1 filter paper. Before determination, two dilutions of the sample were prepared. First volume with potassium chloride buffer, pH 1.0, and second with sodium acetate buffer, pH 4.5. Samples were diluted 12.5 times in pH 1.0 and pH 4.5, and then measured at 520 and 700 nm using a spectrophotometer (Biochrom Libra S22, England). Absorbance of the anthocyanin pigment, expressed as cyanidin-3-glucoside, was calculated as follows:

$$\text{Anthocyanin content} = (A \times MW \times DF \times 1000) / \epsilon \times L$$

where A = (A_{520 nm} - A_{700 nm})_{pH 1.0} - (A_{520 nm} - A_{700 nm})_{pH 4.5}, MW = 449.2 g/mol for molecular weight of cyanidin-3-glucoside, DF = the dilution factor, ϵ = 26,900 molar absorbance, and L = 1 cm for cell path length.

2.3.2 Antioxidant capacity

Antioxidant capacity was determined by free radical scavenging activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). Brown rice was ground and then dried at 75 °C for 48 h; about 0.1 g of the rice flour was transferred into each test tube with 10 mL of the methanol solvent. The extract was shaken on an orbital shaker (IKA KS 250 B) for 30 min. The solution in each tube was separated by centrifugation at 10,000 rpm for 10 min, and filtered through a 0.22 μ m Nylon syringe filter. DPPH radical scavenging activity was evaluated following Amarowicz et al. [11], with some modifications. Briefly, 0.3 mL of the sample extract was transferred into a test tube, and 1.6 mL methanol and 0.5 mL of 0.1 mM DPPH solution was added. Blanks of the extracts were performed using 2.1 mL of methanol, without DPPH solution. The mixtures were shaken, incubated in the dark at room temperature for 20 min, and measured with a spectrophotometer at 517 nm. The DPPH radical scavenging activity was calculated using a calibration curve made using Trolox concentrations. The DPPH radical scavenging activity (%) of samples and standard (Trolox) were calculated as follows:

$$\text{DPPH-scavenging activity (\%)} = [(AC - AS / AC) \times 100]$$

where AC = the absorbance of control and AS = the absorbance of sample. The DPPH scavenging activity was expressed in terms of mg trolox/100 g dry flour.

For FRAP analysis, a modified method of Benzie and Strain [12] was used. Briefly, 2.0 mL of extracted solution was transferred into a 25 mL volumetric flask, and 20 mL of 0.1 M sodium acetate (pH 4.0),

0.5 mL of 0.5 % (w/v) phenanthroline, and 0.5 mL of 0.3 mM Fe (III) were added. Blanks of the extracts were performed using samples as above, except 0.5 % (w/v) phenanthroline was not added and samples were diluted with 0.1 M sodium acetate (pH 4.0). After incubating in a 37 °C water bath for 20 min, the absorbance at 510 nm was measured. The results were calculated from a standard curve prepared with known concentrations of Fe (II), and were expressed as μmol of Fe (II)/100 g of dry flour.

2.4 Statistical Analysis

All statistical analyses were carried out using analysis of variance (ANOVA) (SX window), followed by LSD comparisons tests, at $p < 0.05$. Correlation between

anthocyanin content and antioxidant capacity was determined.

3. RESULTS

3.1 Anthocyanin Content

Anthocyanin content varied significantly among the four purple rice genotypes during storage for six months ($p < 0.05$) (Table 1). The content fell by 42 and 8% in the first month of storage in Kum 7677 and Kum Fang, respectively, the two genotypes with high anthocyanin content, and by six months, the content had declined a further 17% and 19%, respectively. By contrast, there was little change in the two genotypes with low anthocyanin content (Kum Doi Saket and Kum Wiengsa) over the entire six months.

Table 1. Anthocyanin contents of four purple rice genotypes at different storage temperatures during six months.

Storage time (month)	Storage temperature (°C)	Genotype				Mean
		Kum 7677	Kum Fang	Kum Doi Saket	Kum Wiengsa	
0		54.68 a	40.60 b	12.87 ghi	13.25 ghi	30.35
1	-25	38.83 c	37.55 c	13.53 ghi	13.52 ghi	24.35
	RT	31.24 c	37.44 c	11.85 ij	11.71 ij	23.06
	60	30.98 c	37.43 c	11.53 ij	14.45 g	23.59
	mean	31.68	37.47	12.30	13.23	23.67
6	-25	26.45 e	32.74 d	12.06 hij	14.25 gh	21.38
	RT	27.89 e	32.32 d	12.01 hij	12.16 ghi	21.10
	60	24.31 f	26.40 e	10.03 jk	9.16 k	17.48
	mean	26.22	30.47	11.37	11.86	19.99
Genotype (G)					**	
Storage temperature (T)					**	
Storage time (ST)					**	
G × T × ST					**	
LSD (0.05) (G × T × ST)					2.37	

** indicates significant difference at $P < 0.01$. RT indicates room temperature.

Storage temperature affected the anthocyanin content ($p < 0.05$), and this was evident after six months of storage (Table 1). Storage at 60 °C reduced the average anthocyanin content by 42%, compared to the 30% loss for storage at -25 °C or room temperature. Furthermore, storage temperature affected the anthocyanin content differently in brown and paddy forms ($p < 0.05$) (Table 2). In the two-high

anthocyanin genotypes, brown rice had higher anthocyanin content than paddy rice at all storage temperatures. By comparison, in the two low-anthocyanin genotypes, the anthocyanin content was higher in brown rice stored at 60 °C, but not at the other two temperatures. At -25 °C, the anthocyanin content was higher in paddy rice in Kum Doi Saket, but there was no difference in Kum Wiengsa.

Table 2 Anthocyanin contents of four purple rice genotypes during storage at 3 different temperatures in brown and paddy rice forms.

Storage temperature (°C)	Grain form	Genotype			
		Kum 7677	Kum Fang	Kum Doi Saket	Kum Wiengsa
-25	brown rice	36.03 bc	36.60 ab	11.93 mn	13.38 jkl
	paddy rice	33.64 fgh	35.12 cde	13.49 jk	13.74 j
RT	brown rice	35.56 bcd	37.25 a	11.80 mn	12.59 klm
	paddy rice	33.28 h	34.01 efgh	12.71 jklm	12.63 jklm
60	brown rice	34.42 efg	34.67 def	12.27 lmn	13.40 jk
	paddy rice	31.79 i	33.47 gh	11.16 n	12.00 mn
Storage temperature (ST)		**			
Grain form (F)		**			
Genotype (G)		**			
ST × F × G		**			
LSD (0.05) (ST × F × G)		1.12			

** indicates significant difference at $P < 0.01$. RT indicates room temperature.

3.2 Antioxidant Capacity

The antioxidant capacity, both DPPH radical scavenging activity and ferric reducing antioxidant activity power (FRAP), declined in all purple rice genotypes during storage for six months ($p < 0.05$) (Figure 1A, B). The FRAP activity fell to low levels within

the first two months, whereas the DPPH activity declined continuously over the first five months. There was a significant relationship between the antioxidant capacity as measured by DPPH and FRAP during the first three months of storage among the four rice genotypes ($R^2 = 0.79$, $p < 0.05$).

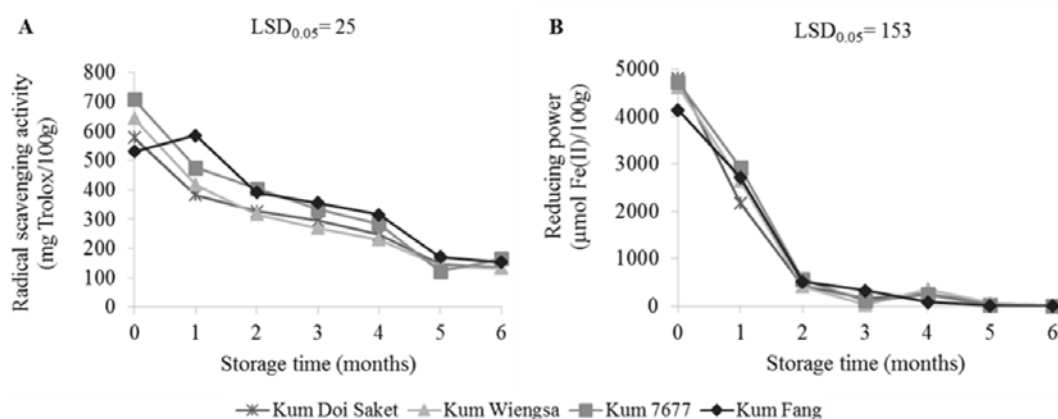


Figure 1. The DPPH radical scavenging activity (DPPH) (A) and ferric reducing antioxidant activity power (FRAP) (B) of four purple rice genotypes during storage for six months.

The effect of storage temperature and grain form on the antioxidant capacity in purple rice grain among the four genotypes was explored using the DPPH data. There was an interaction between storage temperature and grain form on antioxidant capacity ($p < 0.05$) (Table 3). In the two low-anthocyanin genotypes, Kum Doi Saket and Kum Wiengsa, brown rice had higher antioxidant capacity than paddy rice at all storage temperatures, except for Kum Doi Saket at 60 °C, where the antioxidant capacity did not differ between storage forms. Results

were similar for the two genotypes with high anthocyanin, except for Kum Fang at -25 °C and room temperature, where there was no difference in antioxidant capacity between the brown and paddy rice. There was a positive correlation between anthocyanin content and antioxidant capacity in the two high-anthocyanin genotypes (Kum 7677 and Kum Fang) ($R^2 = 0.53$, $p < 0.05$), but the relationship was not observed in the two low-anthocyanin genotypes (Kum Doi Saket and Kum Wiengsa) ($R^2 = 0.039$, $p > 0.05$) (Figure 2).

Table 3. The DPPH radical scavenging activity (DPPH) of four purple rice genotypes after storage at 3 temperatures in brown and paddy rice forms.

Storage temperature (°C)	Grain form	Genotype			
		Kum 7677	Kum Fang	Kum Doi Saket	Kum Wiengsa
-25	brown rice	410.4 a	383.0 b	348.1 de	355.2 cd
	paddy rice	367.5 bcd	376.6 bc	277.5 i	308.1 gh
RT	brown rice	361.4 bcd	369.4 bcd	358.1 cd	317.7 g
	paddy rice	331.2 efg	345.6 def	284.3 hi	284.2 hi
60	brown rice	353.1 cde	349.6 de	271.5 i	318.1 g
	paddy rice	316.9 g	323.6 fg	271.3 i	261.9 i
Storage temperature (ST)		**			
Grain form (F)		**			
Genotype (G)		**			
ST × F × G		**			
LSD (0.05) (ST × F × G)		41.5			

** indicates significant difference at $P < 0.01$. RT indicates room temperature.

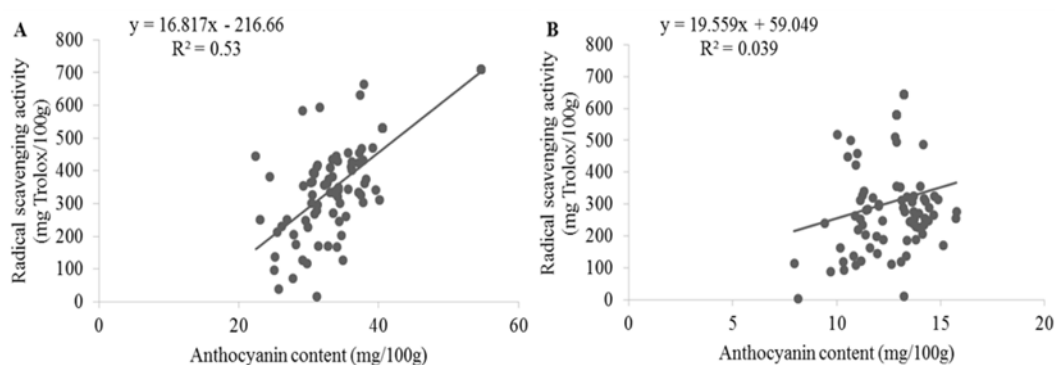


Figure 2. Relationship between anthocyanin content and the DPPH radical scavenging activity (DPPH) among the 2 high (A) and 2 low (B) anthocyanin content of purple rice genotypes ($n = 84$).

4. DISCUSSION

This study demonstrated that both storage temperature and grain form can differentially influence both the anthocyanin content and antioxidant capacity of purple rice genotypes. Anthocyanin has been reported to act as a major anti-oxidative compound in plant tissues, which can protect against oxidative damage implicated in a range of diseases, including cardiovascular ailments and hypercholesterolemia [13-14]. In this study, anthocyanin and antioxidant capacity degradation increased with increasing storage time which was in contrast with the previous study observed slightly decreased anthocyanin in both non-glutinous black and red rice, while antioxidant capacity and carotenoid were increased during storage for four months [15]. Thus, anthocyanin may not be the only major compounds act as an anti-oxidative substance, but other compounds such as flavones, phenols, sterols, tocopherols, γ -oryzanol, amino acids and essential oils, which were not measured in the current study, are also involved as in previously reported [3]. However, this study is the first to show that there is considerable loss in anthocyanin content during rice grain storage depending on several factors such as genotype, storage time and temperature and grain form.

This loss during storage may be due to oxidation cleavage of covalent bonds or increased oxidation and causes in changing of the levels of polymeric pigments with temperature and storage time [16-17] which should be further investigated this reaction from the current study.

The highest storage temperature (60 °C) decreased the stability of anthocyanin and antioxidant capacity in both paddy and brown rice among the four glutinous purple genotypes that were tested and higher remained when stored as brown rice, which there are no previous investigations of this phenomenon in stored rice. A study reported on the effect of temperature during storage that the paddy rice samples stored at 22 °C was found to have higher stability of anthocyanin, γ -oryzanol and vitamin E compared with higher temperature at 30 °C [18]. In contrast, no effect of storage time and temperature was found on the anthocyanin content of non-glutinous rice stored at 20 - 40 °C for four months [15]. The degradations of anthocyanin and antioxidant capacity at high temperature was reported to depend on enzymatic degradation of starch by increasing reducing sugar content which usually occurring during storage in association with the starch properties

independently from grain pericarp color [19]. Therefore, variation of the starch property such as ratio between amylopectin/amylose would differently effect on the degree of degradation in some beneficial compounds of rice grain during storage, especially at high temperature. The stability of anthocyanin at low temperature is significant for grain storage, as presumably enzymic and other oxidation processes that cause discoloration will be repressed. It is remain to further investigate whether the differences in the stability of anthocyanin under high-temperature storage between the Thai purple rice genotypes reflects differences in the amount of enzyme activation, the ratio of starch to protein, or other metabolic factors in the grain. In this study, anthocyanin decreased much more in the high-anthocyanin genotypes than the low-anthocyanin genotypes at all storage temperatures, but especially so at high temperature. Thus, it seems likely that enzyme activation is higher in the high-anthocyanin genotypes, affecting the stability of anthocyanin during storage. Whether there are purple rice genotypes with high anthocyanin content that are not susceptible to pigment breakdown during storage at ambient temperatures requires investigation.

In this study, the anthocyanin content decreased less during storage in the brown rice than the paddy rice in most genotypes. The stability of anthocyanin has been reported to be affected by many factors, including pH, temperature, light, oxygen, metal ions, sugar and enzymes [20]. It is likely that the greater loss of anthocyanin in paddy rice is due to the activities of enzymes, such as polyphenol oxidase and peroxidase, which are located in the husk [21-22]. Higher phenolic acids was reported in the husk (478 mg/100g) compared with in the bran (178 mg/100g), which was degraded by polyphenol oxidase [23], the similar reaction

was observed between polyphenol oxidase and anthocyanin in red sorghum and lettuce [24-25]. Thus, in the current study, the high concentration of enzymes may reflect degradation of anthocyanin and antioxidant capacity in the surface layer of brown rice enclosed by the husk. In addition to further understanding the storage conditions that enhance the anthocyanin content of brown rice, attention should also be given to whether the anthocyanin content can be optimized during parboiling, with its high-temperature soaking and steaming.

Storage not only decreased the anthocyanin in purple rice, but also substantially reduced the antioxidant capacity. The correlation between the DPPH and FRAP assays in the first three months of storage accorded with a previous study on rice affected by salt stress [26]. The stable DPPH is widely using to evaluate the reducing ability of antioxidants toward DPPH, which can be evaluated by electron spin resonance or by measuring the decrease in its absorbance. However, as FRAP measures only the reducing ability based upon the ferric ion, it may not be relevant to antioxidant activity mechanically and physiologically [27]. In the current study, the FRAP values dropped close to zero after three months of storage, which contrasted with the responses observed for DPPH over the same period. The antioxidant compounds in rice have been reported to be related to phenolic acids, proanthocyanidins, γ -oryzanol, tocopherols, tocotrienols, flavonoids and phytic acid, which can contribute to oxidative degradation during storage [24]. The reduction of free phenolic acids in brown rice was reported to correlated with the reaction between phenolic acids and unsaturated fatty acids that located in cell wall remnant by esterification and etherification led to greatly effect on reduction in antioxidant

capacity of purple rice during storage at high temperature which should play be more attention in the further investigation [28].

The effect of storage condition on the stability of antioxidant capacity was quite similar to the change in anthocyanin content. Thus, anthocyanins contribute substantially contribution to the antioxidant capacity in rice grain tissues. This is also suggested by the correlation between the DPPH and anthocyanin levels in the grain of the high-anthocyanin genotypes. The correlation was not found among the low-anthocyanin genotypes, as the antioxidant capacity was similar between the low- and high-anthocyanin genotypes. Thus, some other compounds and/or elements may also act as an antioxidant. One possibility is Zn, as the antioxidant capacity varied significantly with Zn concentration in purple rice varieties [29]. The individual effects of anthocyanin and Zn on antioxidant capacity in purple rice requires further investigation.

5. CONCLUSIONS

Storage conditions affected the stability of anthocyanin content differently among the low- and high-anthocyanin rice genotypes. The stability of anthocyanin during storage was reflected by the antioxidant capacity, especially among the high-anthocyanin genotypes. Low-temperature storage helped delay degradation of anthocyanin and antioxidant capacity compared with high-temperature storage. Storage in the brown rice form may delay enzyme activities, thus preserving anthocyanin and antioxidant capacity, to the benefit of rice consumers.

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