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Full Length Article

Effect of Polishing Time on Distribution of Monomeric Anthocyanin, Iron and Zinc Content in Different Grain Layers of Four Thai Purple Rice Varieties

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Abstract

This study aimed to establish how distribution of nutrients might vary in surface grain layers of purple rice by evaluating their monomeric anthocyanin, Fe and Zn concentration affected by polishing. Concentration of monomeric anthocyanin, Fe and Zn, grain dimension and pigmentation intensity of 4 purple rice varieties (KD, high monomeric anthocyanin; KH, high Fe; LP, high Zn- monomeric anthocyanin; BI, low monomeric anthocyanin-Fe-Zn) from Thailand was determined after polishing five times. Pigmentation reduced in all varieties after 15 s polishing, while monomeric anthocyanin concentration declined in BI and KD but increased in KH and LP. Monomeric anthocyanin ultimately declined in all varieties with longer polishing, but there was no correlation between monomeric anthocyanin concentration and pigmentation intensity at 0 to 30 s. Micronutrients Fe and Zn concentration was increasingly depressed with longer polishing, although the effect on Zn was relatively less. Monomeric anthocyanin, Fe and Zn were distributed differently in successive layers of rice grain removed by polishing, with major varietal difference for monomeric anthocyanin. In some varieties, highest monomeric anthocyanin concentration was in the outer-most grain layer, but was richest below the surface in others. The highest concentration of Fe and Zn were also in outer-most layer, although more Zn was distributed deep in endosperm and most of Fe was removed after 30 s. Such variation should be taken into consideration in efforts to retain or recover potentially valuable nutrients from the grain of purple rice.

Keywords: Monomeric anthocyanin; Iron; *Oryza sativa*; Purple rice; Zinc

Introduction

Rice with pigmented pericarp is a common element of Asia's rice culture. Varieties with various shades of purple are called 'purple' or 'black' rice to differentiate from ordinary, non-pigmented rice with colorless or off-white pericarp. Those with reddish pericarp, not to be confused with 'red rice' contamination of grain with sub-standard quality from weedy or wild rice, are somewhat rarer. Reports on black and purple rice varieties have come from all over Asia (Appa Rao *et al*., 2006; Ahuja *et al*., 2007). The Asian purple rice is predominantly of the waxy endosperm type (Chaudhary and Tran, 2001; Appa Rao *et al*., 2006), which become sticky and glutinous rice when cooked. Pigmented rice has long been part of Asia's traditional pharmacopoeias. In Korea, black rice is considered a health food (Park *et al*., 2008). Pigmented rice is used in Chinese traditional medicine to prevent anemia and improve blood circulation, kidney function and eyesight (Deng *et al*., 2013). Porridge made from black rice is given to aid recovery of invalids; one Chinese variety is known as "healing of broken bones" (Chaudhary, 2003). Claims of medicinal properties of purple rice in Thailand include stopping bleeding after childbirth, bringing down fever to curing skin disease and diarrhea (Kaladee, 2011).

Purple rice is known under local name of *Khao Kam* (dark colored rice) in Lao PDR (Appa Rao *et al*., 2006) and northern Thailand, for the dark color of its unpolished 'brown rice', de-husked caryopsis, with embryo, pericarp and aleurone still intact, the form normally used. Nutritional and pharmacological properties of purple rice have been elucidated in many reports focusing on its content of many nutrients including compounds with capacity to neutralize the toxic effects of oxygen radicals, or antioxidative property. Although some of these studies were based on analysis of extracts from brown rice (Park *et al*., 2008; Yodmanee *et al*., 2011), others (Han *et al*., 2004; Saenjum, 2012) have clearly shown that most compounds of

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interest are in the 'bran' section, surface layers of de-husked caryopsis (pericarp, the aleurone and some sub-aleurone cells) plus the embryo which are removed by polishing in the milling process. Removal of bran by polishing or buffing to produce 'white rice' generally preferred by rice eaters has been clearly shown to depress Fe and Zn concentration (Prom-u-thai *et al*., 2007; Saenchai *et al*., 2012).

Although, pigmented rice has traditionally been used for preparation of deserts and special foods for festivities and religious offerings, and rarely consumed as staple, there is now increasing interest in it as a delicacy and as a source of various nutrients and compounds with medicinal and pharmacological potential. Numerous modern high yielding black rice varieties have been developed in China (Chaudhary, 2003). In Thailand, where special quality characteristics continue to be discovered in local purple rice landraces and new varieties developed (Prom-u-thai and Rerkasem, 2001; Phengrat and Jearakongman, 2009; Kaladee, 2011; BRRD, 2013), purple rice is a common part of the country's retail rice market, with prices many times higher of ordinary rice. The objective of this study was to evaluate how concentration of monomeric anthocyanin, Fe and Zn are affected by different intensity of polishing, and to shed light on localization of nutrients in successive grain layers of four different purple rice varieties. These results may help in milling and processing to modify and optimize recovery or retention of potentially useful novel compounds.

Materials and Methods

Plant Culture

Four purple rice varieties (Kam Hom Morchor, KH; Kam Doi Saket, KD; Luem Pua, LP; Bieisu, BI) were grown together in field in wet season (June-November), 2012, at Chiang Mai University, Thailand. The soil was sandy loam of Sansai series. Rice seeds were soaked in water overnight and incubated moist until germinated and grown for 30 days in a seedbed. Single seedlings were transplanted into hills at 25 × 25 cm spacing. Nitrogen fertilizer was applied using 75 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.

Sample Preparation

The rice seed was de-husked to produce unpolished rice (0 s) with a laboratory husker (Model P-1 from Ngek Seng Huat Co. Ltd., Thailand). Three replicates of 50 g sub-samples of unpolished rice of each variety were milled separately at 15, 30, 45 and 60 s with a laboratory polisher (Model K-1 from Ngek Seng Huat Co. Ltd., Thailand). The metal parts of both de-husker and polisher were carefully cleaned to avoid Fe and Zn contamination between samples. Grain dimension (width × length × thickness) were measured on fifty grain from each sample of 0, 15, 30, 45 and 60 s polished rice with a digital caliper, putative volume of the grain was derived by multiplication of grain width, length and thickness, to determine the extent of grain surface removal by different degree of polishing. Pigmentation intensity was measured with a chroma meter (model CR-300, Minolta, Osaka, Japan), with the L value representing degree of light or darkness of sample (L = 100, light; L = 0, dark). Whiteness was evaluated in a milling meter (Satake, Japan).

Chemical Analysis

Samples were oven dried at 75°C for 72 h before Fe and Zn determined using a Hitachi Z-8230 atomic absorption spectrophotometer (Zarcinas *et al*., 1987). Monomeric anthocyanin was determined by the pH-differential method (Escribano-Bailón *et al*., 2004; Giusti and Wrolstad, 2001). Briefly, 2.5 g freeze dried samples were extracted in double deionized water (DDI) at 50°C for 30 min. The extracted solution was filtered with filter paper before preparing two dilutions. Volume of one was adjusted with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5). Each dilution was allowed to equilibrate for 15 min. Absorbance of the first dilution was measured at 520 and second at 700 nm, against a blank cell filled with distilled water. Absorbance readings were made after 15 min and completed within 45 min. The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$$

The monomeric anthocyanin pigment concentration in the original sample was determined with the following formula:

$$\text{Monomeric anthocyanin content (mg L}^{-1}\text{)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

The concentration was converted to mg of total anthocyanin content /100 g sample (MW, molecular weight of cyanidin-3-glucoside = 449.2, DF = dilution factor; ε, molar absorptivity of the pigment = 26,900).

Data Analysis

The data were subjected to analysis of variance (ANOVA) and means were separated at $P< 0.05$ by the least significant difference (LSD) test. Certain sets of data were also subjected to correlation and regression analysis.

Results

Unpolished (0 s) grain of four rice varieties differed significantly in nutrient contents, intensity of pigmentation and dimension (Table 1). These purple rice varieties ranged from moderately high to high Fe and Zn, with KH being highest in Fe and LP highest in Zn. Monomeric anthocyanin concentration of unpolished rice grain was highest in KD, slightly lower in LP, much lower in KH and very little in BI.

Table 1: Dimension of unpolished grain (0 s) of 4 purple rice varieties, their iron, zinc and anthocyanin content and degree of pigmentation

Attribute	Variety†			
	KH	KD	LP	BI
Grain dimension (width × length × thickness = putative volume)				
Width (mm)	3.14 d	2.63 b	2.86 c	2.51 a
Length (mm)	6.01 a	6.48 c	7.82 d	6.21 b
Thickness (mm)	2.04 d	1.77 a	1.89 c	1.83 b
Putative volume (mm^3)	38.40 b	30.10 a	42.20 c	28.50 a
Nutrient concentration‡				
Iron (mg Fe kg^{-1})	40.00 c	18.00 b	19.00 b	14.00 a
Zinc (mg Zn kg^{-1})	38.00 a	35.00 a	55.00 b	34.00 a
Anthocyanin (mg 100 g^{-1})	5.70 b	18.90 d	15.70 c	1.30 a
Degree of pigmentation§				
L value	25.60 a	35.20 b	39.20 c	50.80 d
Whiteness (%)	5.10 a	5.80 a	6.10 a	8.50 b

†Different letters indicate significant difference (by LSD at P <0.05) between variety within each row (n = 3);‡On dry weight basis;§The *L* value was measured with a chroma meter, representing grain brightness, ranging from 0 (dark) to 100 (light). Whiteness was measured with milling meter

Table 2: Changes in putative grain volume and iron, zinc and anthocyanin concentration by polishing in 4 purple rice varieties

Variety (V)	Polishing time (T)				
	15 s	30 s	45 s	60 s	Mean
Putative volume depressed (%)†					
KH	13.70	28.20	28.70	31.20	25.50 A
KD	15.70	27.60	31.00	33.50	27.00 A
LP	23.00	33.60	37.10	40.90	33.60 B
BI	16.80	23.50	27.60	29.60	24.40 A
Mean	17.30 A	28.30 B	31.10 BC	33.80 C	
Significant effects‡§: V, $P < 0.001$; T, $P < 0.001$; Not significant $P < 0.05$					
Zinc concentration depressed (%)†					
KH	15.20	31.30	27.90	31.20	27.50 B
KD	17.10	18.50	20.10	23.80	18.30 A
LP	19.10	18.20	21.70	22.70	20.20 AB
BI	8.10	15.60	19.80	15.80	14.80 A
	14.10 A	20.90 A	22.40 B	23.40 B	
Significant effects‡§: V, $P < 0.01$; T, $P < 0.01$; V×T Not significant $P < 0.05$					
Iron concentration depressed (%)†					
KH	31.30 aA	87.40 bB	94.80 cC	97.60 cB	77.80
KD	48.30 aC	66.30 bA	85.30 cB	87.20 cA	71.80
LP	37.10 aAB	69.20 bA	76.50 bA	89.00 cA	67.90
BI	42.50 aBC	68.80 bA	83.60 cB	88.50 cA	70.90
Mean	39.80	72.90	85.10	90.60	
Significant effects‡§: V, $P < 0.001$; T, $P < 0.001$; V × T, $P < 0.001$					
Anthocyanin concentration depressed (%)†					
KH	-148.70 aA	47.70 bB	83.90 bcA	91.80 cA	18.70
KD	57.90 aC	73.70 aB	87.60 aA	99.60 bB	79.70
LP	-43.20 aB	23.40 bA	72.30 cA	90.60 cA	35.80
BI	90.50 aC	100.00 bC	100.00 bB	100.00 bB	97.60
Mean	-10.90	61.20	86.00	95.50	
Significant effects‡: V, $P < 0.001$; T, $P < 0.001$; V × T, $P < 0.001$					

†Compared with 0 s. Significant difference by $LSD_{p<0.05}$ (of the arcsine values where relevant) designated by different lowercase letters in the same row and uppercase letter in the same column;‡By analysis of variance §After arcsine transformation

Measured by the *L* value of unpolished grain, the variety KH had darkest color, followed by KD, LP and BI, respectively. The *L* value was closely associated with whiteness (r = 0.95, $P < 0.01$), although KH was significantly less white and other 3 varieties were relatively less distinguishable when measured in whiteness. Monomeric anthocyanin concentration of 0 s rice did not correlate significantly ($P < 0.05$) with intensity of pigmentation, measured either in the *L* value or whiteness.

In spite of significant difference in the original grain dimension, putative volume of rice varieties was depressed to similar extent by polishing (Table 2). Most of the depression in grain volume occurred after 30 s, 17% at 15 s and 28% at 30 s, but only a little more with longer polishing. Polishing affected Fe, Zn and monomeric anthocyanin concentration and putative volume of grain in different rice varieties differently (Fig. 1). Differential effects of polishing on the nutrients can be seen in changes of their concentration over polishing time (Table 2). Concentration of both Fe and Zn declined with longer polishing, but the effect on Zn was much milder than on Fe. Without significant difference among the varieties ($P< 0.05$), the biggest Zn depression was 15% at 15 s, followed by another 7% decline at 30 s, and with little further effect of longer polishing. After 60 s, the grain Zn concentration still averaged three quarters of unpolished grain. Iron concentration was depressed more strongly, with sharper decline in high Fe variety KH, which was brought to the same level as in other 3 varieties after 30 s, and by 60 s polishing almost all of Fe removed in all 4 varieties (Fig. 1).

Monomeric anthocyanin concentration was affected by polishing differently from Fe and Zn, with different effects among the rice varieties. After 15 s, monomeric anthocyanin concentration was depressed by 58% in KD, 90% in BI but increased by 43% in LP and 145% in KH (Table 2). Variation in the effect of polishing on monomeric anthocyanin of the rice varieties continued to 30 s, when 76% of original concentration still retained in LP, compared with 52% in KH, only 26% in KD and none in BI. Relative richness in monomeric anthocyanin of rice varieties was thus markedly altered by polishing, with KD, the variety with the highest monomeric anthocyanin at 0 s, becoming the second lowest after 15 - 30 s. Intensity of pigmentation became increasingly faded with longer polishing time, as indicated by increasing *L* value, but at varying rates among the rice varieties (Fig. 2). The fading was most rapid in KH, the variety which had the most intense pigmentation at 0 s became the least dark after 60 s. Although there was a general trend of declining anthocyanin concentration with fading pigmentation over longer polishing, intensity of pigmentation of 0 – 30 s rice did not correlate significantly ($P < 0.05$) with monomeric anthocyanin concentration.

Discussion

Anthocyanin is considered the primary bioactive compound in purple rice (Hiemori *et al.*, 2009; Deng *et al.*, 2013). Purple rice varieties from southern Thailand were found to contain more than ten times the concentration of anthocyanin in reddish brown varieties (Yodmanee *et al.*,

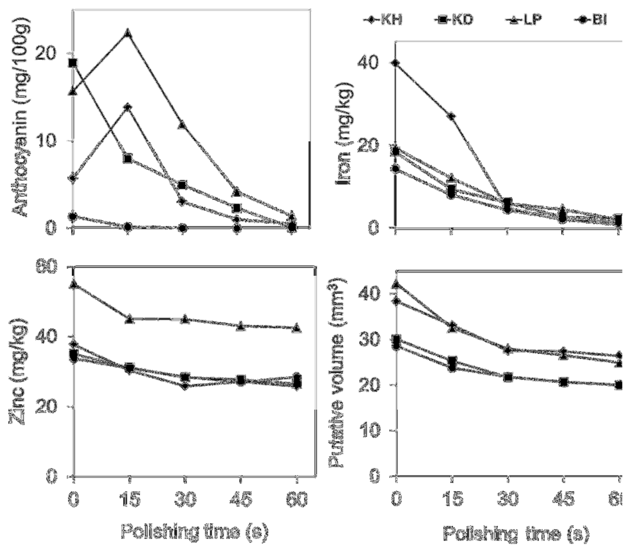

Fig. 1: Effect of polishing on anthocyanin (upper left), iron (upper right) and zinc (lower left) concentration and putative grain volume (lower right, derived from multiplication of grain width, length and thickness) in 4 purple rice varieties. (n = 3)

Significant effects by analysis of variance ($LSD_{p<0.05}$ in brackets)			
	Variety (V)	Polishing time (T)	V×T
Zinc	$P < 0.001$ (1.4)	$P < 0.001$ (1.5)	$P < 0.05$ (3.1)
Iron	$P < 0.001$ (1.3)	$P < 0.001$ (1.4)	$P < 0.001$ (2.9)
Anthocyanin	$P < 0.001$ (1.1)	$P < 0.001$ (1.2)	$P < 0.001$ (2.4)
Putative volume	$P < 0.001$ (0.9)	$P < 0.001$ (1.0)	$P < 0.001$ (1.9)

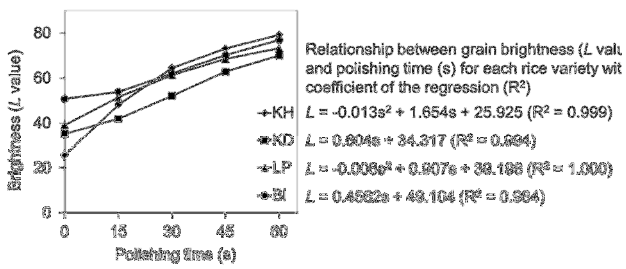

Fig. 2: Fading intensity of pigmentation by longer polishing, measured as degree of brightness, the *L* value on a chroma meter, ranging from 0 (dark) to 100 (light) in 4 purple rice varieties. (n = 3)

Significant effects by analysis of variance ($LSD_{p<0.05}$ in brackets)		
Variety (V)	Polishing time (T)	V×T
$P < 0.001$ (1.0)	$P < 0.001$ (1.1)	$P < 0.001$ (2.2)

2011). This study has shown that monomeric anthocyanin concentration cannot always be predicted from intensity of pigmentation of the unpolished and lightly polished rice grain. In other plant species, lack of correlation between monomeric anthocyanin content and optical quality has been explained in different ways. For example, the amount of red light reflected from anthocyanin rich senescing leaves, and hence intensity of their red pigmentation was reported to be more dependent on distribution and content of chlorophyll instead of anthocyanin (Gould, 2004). As anthocyanin in red grapes and young wines disappears during fermentation and aging, the deep color characteristic of aged red wines has been shown to be the product of new wine pigments derived from complexes of monomeric anthocyanin with other molecules, especially with tannin (He *et al*., 2012; Brouillard *et al*., 2003). Pigmented rice varieties are generally very rich in tannin, with concentration in the bran 40-80 times than in bran of non-pigmented rice (Goffman and Bergman, 2004). Although unpolished grain of some Thai purple rice varieties have high in both monomeric anthocyanin and polyphenols (Yodmanee *et al*., 2011), it remains to be explored if complexes of anthocyanin with tannin or other phenols are part of the explanation of very low anthocyanin content of some purple rice varieties as found here.

Compared with published values for non-pigmented rice from other rice growing countries of Asia as well as Thailand (Saenchai *et al*., 2012), unpolished grain of the purple rice in this study ranged from moderately high to high Fe and Zn, with KH being the highest in Fe and LP highest in Zn. Polishing depressed Fe and Zn concentration in these purple rice varieties as reported for non-pigmented rice. The 30 s polishing produces regular milled rice in popular non-pigmented Thai rice varieties depresses the concentration of Fe by 46±12% and Zn by 31±15% (Saenchai *et al*., 2012). The sharp decline in Fe concentration after 30 s polishing indicated that Fe was more concentrated in surface layer of purple rice grain, especially the high Fe variety KH, with 87% decline of grain Fe after 30 s. Zinc in purple rice was distributed deeper in the endosperm, in the same way as the higher Zn varieties of non-pigmented rice, with Zn concentration of the purple rice depressed by only 21±7% at 30 s and 23±6% at 60 s. A previous study on milling depression in variety KD also investigated that anthocyanin, γ-oryzanol, and γ-tocopherol decreased by 74.49, 55.35, and 70.36%, respectively, after 10 s of milling (Laokuldilok *et al*., 2013). However, this study was carried out with an only variety (KD), which would be a variation of decreasing among different rice varieties.

The pattern of monomeric anthocyanin distribution found in the varieties KD and BI, with the highest concentration in outer-most grain layer, was somewhat similar to Fe and Zn. In these purple rice varieties, monomeric anthocyanin concentration was increasingly depressed with longer polishing. However, a different pattern of monomeric anthocyanin distribution was indicated with significant elevation of monomeric anthocyanin concentration by 15 s polishing in KH and LP varieties. This varietal difference in monomeric anthocyanin distribution was not related to concentration in unpolished grain, may possibly influence pigmentation intensity and optical property of purple rice. In non-pigmented rice, the form of grain preferred by consumer depend on white rice is produced by removal of bran. But for pigmented rice, bran may need to be considered in its different component tissues which include the pericarp, the aleurone layer, sub-aleurone cells and the embryo. The pericarp and embryo, components of the bran removed at 15 s constituted 17% depression of the putative volume, apparently contained little monomeric anthocyanin. In addition to monomeric anthocyanin, the bran of pigmented rice also contains other novel and potentially useful compounds, e.g. anti-oxidative phenols (Goffman and Bergman, 2004) and γ-oryzanol (Juliano *et al.,* 2005). Efforts to exploit such novel nutritional and pharmaceutical quality in pigmented rice, either in evaluation and screening of genotypes or in processing, may benefit from understanding of their distribution in the grain which could be affected by the milling process.

Conclusion

This study indicated that anthocyanin, Fe and Zn were distributed differently in successive layers of rice grain removed by polishing, with major varietal difference for the anthocyanin. In some varieties the highest anthocyanin concentration was in the outer-most grain layer, but was richest below the surface in others. The highest concentration of Fe and Zn were also in outer-most layer, although more Zn was distributed deep in the endosperm and most of Fe was removed after 30 s. These results could help in milling and processing to modify and optimize recovery or retention of potentially useful novel compounds.

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