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Total phenolic content and antioxidant capacity of rice grains with extremely small size

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Whole cereal grains have been received increasingly attention by consumers due to their potential health benefits because of their antioxidant capacity, which is probably derived from their high contents of phenolics, flavonoids and other phytochemicals. Here, we reported the contents of phenolics and flavonoids in different rice genotypes with grain size ranging from extremely small to normal size. The smaller grains had higher phenolic content, flavonoid content and antioxidant capacity than the normal and larger grains. The phenolic content had positive correlation with the flavonoid content (*P*<0.001) and the antioxidant capacity (*P*<0.01). The phenolic and flavonoid content had negative correlation with grain length, grain length to width ratio and 100-grain weight (*P*<0.01), but had no relationship with grain width and grain thickness. Thus, the phenolic content could be indirectly predicted by grain length and 100-grain weight. New rice varieties high in antioxidant levels could be obtained by breeding for extremely small grain rice.

Key words: Phenolics, flavnoids, antioxidant capacity, rice grains.

INTRODUCTION

Polyphenol and flavonoid which have antioxidant capacity present in many foods and vegetables are regarded as the functional materials. Regular intake of these phytochemicals can reduce many chronic diseases such as cardiovascular diseases, heart diseases, diabetes, obesity and certain cancers, and improve endothelial function and reduce blood pressure (Liu, 2007; Yawadio et al., 2007; Jonathan and Kevin, 2006). Phenolics exist in any grain crops and vegetables. Over the past few years, there has been an increasing interest in the study of the antioxidant compounds in grains in relation to health benefit because of their antioxidant activity (Butsat et al., 2009; Lai et al., 2009; Shen et al., 2009; Tananuwong and Tewaruth, 2010; Zhang et al., 2010). Rice is one of the most important cereal crops all over the world. With the improvement of people's living standards, functional foods are becoming more and more popular among different countries. It has been reported that corn had the highest total phenolic content and antioxidant activity, followed by wheat, oats and rice (Adom and Liu, 2002). Dehulled red rice showed a total antioxidant capacity more than three times greater than dehulled white rice and its high total antioxidant capacity was essentially characterized by the presence of proanthocyanidins and associated phenolics (Finocchiaro et al., 2007).

The total phenolics and flavonoids in bran part of grains are higher than those in whole-grain flours (Butsat and Siriamornpun, 2010; Goffman and Bergman, 2004; Kong and Lee, 2010; Mattila et al., 2005; Zhang et al., 2010). Black rice bran has higher content of phenolics and anthocyanins, and has higher antioxidant activity when compared to white rice bran (Zhang et al., 2010, Goffman and Bergman, 2004). The radicals scavenging activities of phenolic compounds in rice grain have been well demonstrated in cells or animal models (Chi et al., 2007; Rattanachitthawat et al., 2010). The interest in health benefit of whole grain rice may drive breeding programs further to enhance nutrient dense in the grain. Examples are the transgenic improvement of nutrients of β-carotene (Ye et al., 2000) and folate (Storozhenko et al., 2007) in rice grain. Surveying rice genotypes from a wide collection of germplasm could indentify some rice materials high in the total phenolics, flavonoids contents and antioxidant capacity (Shen et al., 2009). Understanding the genetic bases underlying the natural

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occurring genetic diversity in the phytochemicals in relation to health benefit may help the breeding process (Jin et al., 2009).

In the present study, the total phenolics content, total flavonoid content and the total antioxidant capacity of rice with extremely small grain size were measured; the results showed that those extremely small rice grain had higher phenolics and antioxidant capacity than those of normal or large grain. Thus, we propose that breeding extremely small grain is a way to improve antioxidants to obtain rice with high health benefit.

MATERIALS AND METHODS

Rice materials

A total of 18 non-pigmented rice accessions were harvested in early April, 2009 in Hainan. After being air-dried and stored at room temperature for three months, rice grains were dehusked on a Satake Rice Machine (Satake Co., Japan), then the brown rice grains were ground to pass through a 100-mesh sieve on a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado, USA).

Physical properties

The grain length, grain width and grain thickness were measured with ten grains using a vernier caliper. The 100-grain weight was measured in triplicate from one hundred brown grains. The color of brown rice grain was measured by Minolta Chroma Meter CR-301 (Minolta Co., Osaka, Japan), standardized with calibration plate sets CR-A47 and a white plate. Color measurements were expressed as tristimulus parameters, L^*, a^* and b^*. L^* indicates lightness (100 white and 0 black). a^* indicates redness-greenness and b^* indicates yellowness-blueness. In addition, the chroma (C) value indicates color intensity or saturation, calculated as $C = (a^{*2} + b^{*2})^{1/2}$ and Hue angle was calculated as $H° = \tan^{-1} (b^*/a^*)$, where 0° or 360° = red-purple, 90°= yellow (Bao et al., 2005).

Extraction

Brown rice flours (1 g) of each sample were extracted with 20 ml of 80% methanol for 24 h at room temperature. The procedure was repeated twice. The methanolic extracts were centrifuged at ~4000 g for 15 min and the supernatants were pooled and stored at 4°C.

Total phenolics content

The Folin-Ciocalteu colorimetric method was used to measure the total phenolic content (Bao et al., 2005). Briefly, 200 µl of the extractions were oxidized with 1 ml of 0.5 N Folin-Ciocalteu reagent and then the reaction was neutralized with 1 ml of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY) after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent (mg GAE) per 100 g of flour weight.

Total flavonoid content

Total flavonoid content was determined by a colorimetric method (Bao et al., 2005). 0.5 ml extracts were added to 15 ml polypropylene conical tubes containing 2 ml ddH_2O and mixed with 0.15 ml 5% $NaNO_2$. After reacting for 5 min, 0.15 ml 10% $AlCl_3.6H_2O$ solution was added. After another 5 min, 1 ml 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at 415 nm. Qualification was done using the Rutin as standard and the results was expressed as milligrams of rutin equivalent (mg RE) per 100 g of flour weight.

The total antioxidant capacity

The total antioxidant capacity was determined by a colorimetric method by using the Spectronic Genesys 5 spectrophotometer (Milton Roy, NY) with a little modification (Re et al., 1999; Bao et al., 2005). First, $ABTS^{\cdot +}$ solution prepared and was adjusted with pH to about 0.700 with 80% ethanol at 734 nm. Then, 3.9 ml $ABTS^{\cdot +}$ cation solution was added to 0.1 ml of extracts and mixed thoroughly. The mixture incubated for 6 min at room temperature and tested the absorbance at 734 nm. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µM Trolox equivalents per 100 g dry weight).

Statistics analysis

All the analyses were carried out at least in duplicate and in a randomized order with mean values being reported. Analysis of variance (ANOVA) and correlation analysis of the results were performed in SAS (Software Version 8.1. SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The physical properties of rice samples

Most of the rice accessions used in this study are breeding lines, which differed dramatically in the grain size (Figure 1). The length of selected samples was ranged from 3.28 mm (PF36) to 7.55 mm (BP603), the grain width was ranged from 2.26 mm (BP620) to 3.24 mm (KA005), and the grain thickness was ranged from 1.72 mm (BP608) to 2.28 mm (KA005). The rice accession PF36 is nearly round and smallest grain with 100-grain weight of 1.32 g. Another rice BP305 had a little longer in length than PF36, but it had the lowest 100-grain weight (1.15 g) among all the rice accessions used in this study. KA005 was the plump one with the largest width and thickness in size and had 100-grain weight of 1.97 g. Nine rice accessions had the length between 5 to 6 mm. Two accessions had the length between 6 to 7 mm. Three accessions had the grain length larger than 7.0 mm. The longest one was BP603 (7.55 mm) with 100-grain weight of 2.53 g (Table 1 and Figure 1). Because all rice are brown rice, the color parameters of the grain did not show large variations. The BP620 was the most darkness with L^* value of 54.5. There were other three rice that had L^* smaller than 60, while all other rice had L^* value larger than 60.

From the hue data (H^o), three rice accessions had the

Figure 1. Rice accessions used in the study. The rice was arranged according to its length and with the same order as shown in Table I. The bar = 10 mm.

H^o smaller than 80, while all others larger than 80 (Table 1 and Figure 1).

The total phenolic content and antioxidant capacity

The smallest grain of PF36 and BP305 had the phenolic content of 100.7 and 78.7 mg GAE/100 g, respectively. The rice BP033 with large grains had the lowest phenolic content (42.57 mg GAE/100 g). The flavonoid content among these samples varied from 62.1 mg rutin equivalent (mg RE)/100 g (BP034) to 182.6 mg RE/100 g (PF36). BP289 had the lowest antioxidant capacity (230.1 µM TEAC/100 g), and the PF36 had the highest antioxidant capacity (323.88 µM TEAC/100 g) (Table 1). The genetic diversity in antioxidant levels of white rice was rarely reported before. Shen et al. (2009) reported the wider variations of phenolic content, flavonoid content and antioxidant capacity among a total of 423 white rice, but the rice with extremely small grain was not included. Some other reports only measured the total phenolic, flavonoid contents and antioxidant capacity with few rice accessions (Butsat and Siriamornpun, 2010; Chotimarkorn et al., 2008; Iqbal et al., 2005). The phenolic content and antioxidant capacity of brown rice are higher than the milled rice (Butsat and Siriamornpun, 2010). To date, rare reports focused on the grain size and its relation to the phytochemicals and antioxidant capacity.

Correlation analyses

The correlation coefficients among phenolics, flavonoid, total antioxidant capacity and grain physical properties are listed in Table 2. In general, the phenolic content had a strong positive correlation with the flavonoid content and antioxidant capacity (P<0.01), which was in agreement with many of previous studies such as Chi et al. (2007), Jin et al. (2009), Rattanachitthawat et al. (2010), Shen et al. (2009) and Zhang et al. (2010). The phenolic and flavonoid contents had significant negative correlation with grain length and grain length to width ratio, but not with the grain width and grain thickness (Table 2). They were also significantly correlated with 100-grain weight, which indicated grain length but not grain width and grain thickness determined the grain weight, as also could be seen from the correlation between grain length and 100-grain weight (P < 0.001). The negative correlations between the 100-grain weight and phenolic content, flavonoid content and antioxidant capacity were also reported by Shen et al. (2009). The 100-grain weight and grain length had weak negative correlation with antioxidant capacity, whereas significant correlations between them were also described in Shen et al. (2009). It was noted that the significant correlations might be driven by the two small rice cultivars, because without these two smallest grain accessions, the correlation coefficients of phenolic and flavonoid content with grain grain length were around -0.45 (P > 0.05), and with 100-GW were -0.39 (P > 0.13) and -0.48 (P > 0.05), respectively. It appeared that for accessions having

Table 1. Physical properties and antioxidant capacity of rice accessions.

No.[a]	Accessions	GL (mm)	GW (mm)	GT (mm)	GLtoW	100-GW (g)	*L**	*a**	*b**	*C*	*H*°	PC (mg GAE/100 g)	FC (mg RE/100 g)	TAC (μM TEAC/100 g)
1	PF36	3.28	3.02	2.27	1.09	1.32	60.4	3.8	20.0	20.4	79.1	100.7	182.6	323.9
2	BP305	3.49	2.76	1.74	1.26	1.15	60.7	2.9	19.8	20.0	81.7	78.7	123.9	302.7
3	KA005	4.66	3.24	2.28	1.44	1.97	65.4	2.7	19.1	19.3	82.1	57.3	87.2	246.2
4	BP620	4.76	2.26	1.89	2.11	1.34	54.5	4.9	20.8	21.3	76.7	58.4	113.1	254.5
5	BP605	5.14	3.04	2.15	1.69	2.19	60.5	3.1	19.9	20.2	81.2	62.1	117.9	273.3
6	BP034	5.21	2.96	1.97	1.76	2.17	68.1	2.3	15.3	15.5	80.4	56.1	62.1	276.5
7	BP289	5.32	2.74	1.79	1.95	2.00	63.4	2.8	19.3	19.5	81.8	61.3	87.5	230.1
8	KA002	5.32	3.08	2.15	1.73	2.22	67.9	2.3	18.9	19.0	83.1	49.1	67.5	277.4
9	D007	5.67	2.93	2.16	1.94	2.01	63.7	3.4	20.3	20.6	80.5	63.1	72.6	317.0
10	BP608	5.72	2.31	1.72	2.48	1.60	62.9	1.7	20.4	20.5	85.4	53.0	74.5	281.1
11	D093	5.80	2.84	2.12	2.05	2.10	63.8	2.7	17.2	17.4	81.1	62.6	110.3	244.3
12	BP020	5.86	2.43	1.74	2.42	1.68	56.0	3.5	19.0	19.3	79.6	67.5	102.4	316.5
13	BP341	5.96	2.70	1.88	2.21	2.11	62.8	2.6	19.4	19.6	82.4	51.4	64.7	280.2
14	BY019	6.16	2.66	2.01	2.32	2.20	59.4	3.1	17.8	18.1	80.2	56.2	86.8	260.0
15	D153	6.90	2.86	1.99	2.43	2.11	61.5	2.5	18.7	18.9	82.3	46.6	70.4	231.9
16	BP033	7.04	2.53	2.07	2.78	2.47	58.8	2.7	17.1	17.3	81.2	42.6	67.8	249.4
17	JS056	7.05	2.72	2.15	2.60	2.54	58.9	2.5	18.2	18.3	82.2	56.6	62.4	281.1
18	BP603	7.55	2.57	2.05	2.93	2.53	61.0	2.3	17.4	17.6	82.6	53.6	76.4	261.4
	LSD[b]	0.20	0.12	0.08	0.11	0.024	1.71	0.49	2.53	2.51	2.08	5.81	27.8	24.3

[a] The rice accession was listed according to its length, from short to long. [b]LSD: Least significant difference ($P < 0.05$). Abbreviations: GL, grain length; GW, grain width; GT, grain thickness; GLtoW, grain length to width ratio; 100-GW, 100 grains weight; PC, phenolic content; FC, flavonoid content; TAC, total antioxidant capacity.

Table 2. Correlation coefficients among the physical properties and antioxidant activies.

	GL	GW	GT	GL to W	100-GW	*L**	*a**	*b**	*C*	*H*°	PC	FC
GW	-0.33											
GT	0.01	0.69**										
GLtoW	0.93***	-0.65**	-0.24									
100-GW	0.81***	0.18	0.4	0.58*								
*L**	-0.11	0.66**	0.26	-0.35	0.26							
*a**	-0.42	-0.17	0.07	-0.28	-0.51*	-0.63**						
*b**	-0.46	-0.13	-0.16	-0.32	-0.63**	-0.31	0.44					
C	-0.48*	-0.15	-0.15	-0.33	-0.65**	-0.35	0.50*	1.00***				
H°	0.33	0.11	-0.11	0.23	0.34	0.52*	-0.92**	-0.06	-0.13			

Table 2. Contd.

PC	-0.75***	0.22	0.1	-0.68**	-0.67**	-0.17	0.47*	0.35	0.37	-0.4		
FC	-0.72***	0.12	0.13	-0.62**	-0.68**	-0.34	0.60**	0.41	0.44	-0.50*	0.89***	
TAC	-0.41	0.03	-0.01	-0.33	-0.42	-0.11	0.23	0.32	0.32	-0.16	0.62**	0.37

See Table 1 for abbreviations. *, **, and *** indicated significance at $P < 0.05$, 0.01 and 0.001, respectively.

GL> 4.6 mm and 100-GW > 1.32g, GL and 100-GW were not good predictors of phenolic and flavonoid content.

Conclusion

The rice grains with extremely small size or low 100-grain weight generally had higher phenolic content, flavonoid content and antioxidant capacity than grains with normal or large size. Phenolic content and antioxidant capacity of rice grain are significantly correlated with each other. The phenolic content could be indirectly predicted by grain length and 100-grain weight. Therefore, new rice varieties high in antioxidant levels could be achieved by breeding for extremely small grain rice.

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