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The changes in rice antioxidant properties during storage at different temperature

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INTRODUCTION

Many epidemiological studies indicate thatregular consumption of whole grains or related food products incidences of chronic disease and several forms of cancer[3]. The biologically plausible mechanisms may be due to the scavenging of free radicals by the bioactive phytochemicals present in whole grains[22]. Phytochemical compounds mainly present in whole grains are phenolic acids and flavonoids[2]. The main phenolic acids found in whole grains are ferulic acid, vanillic acid, caffeic acid, syringic acid and *p*-coumaric acid[25], while flavonoids are flavonols, flavan-3-ols, flavones and flavanones[12]. Cereal grains are major food sources worldwide. Whole grains are usually made up of the endosperm, the germ, and the bran of the grain. The endosperm makes up about 80% of the whole grain, while the germ and bran components vary among different grains. Among them, rice (*Oryza sativa* L.) is being consumed as one of the most important staple foods, currently sustains two-thirds of the world's population. Rice is classified according to the degree of milling: brown rice, remains unmilled with just the removal of the hull, while white rice is milled by removing embryo and bran. The consumption of brown rice is becoming more and more popular because of its higher dietary fiber and antioxidants contents compared to white rice. In brown rice, phenolic acids exist in both free and bound forms[16,27], which the later form is believed to exist in grain as ester-liked to cell wall polymers and consist mainly of ferulic acid and its oxidatively coupled dimers[20,23]. Ferulic acid has been known as antioxidant which is effective toward anti-inflammation and inhibition of tumor initiation and as a preservative[1]. There are a number of literature reports on phenolic compounds and their antioxidant activities in cereal, fruit, and vegetable. However, the literature reports on rice is relatively limited[14].

The reducing capacity of antioxidant is an important parameter to evaluate its functional property. Recently, the determination of total antioxidant activity has gained a strong interest as a directionto explore the putative role of antioxidant-rich products in the prevention of degenerative disease and to screenthe grain varieties with potentially positively health benefits. Rice is consumed as cooked rice and only a small amount of rice is used to make ingredients for processed foods. This pattern of usage results in the need to store rice over varying periods. During storage, a number of changes in rice chemical and physical properties occur, which is usually termed rice ageing. Rice ageing commences before harvest and continues. Ageing-induced changes occur in rice composition, pasting properties, thermal properties and texture[26]. However, the study on the effect of storage on brown rice antioxidant activity is still limited in the available rice literature. Thus, the aim of this work was to study the changes intotal phenolic content, total flavonoid content, ferulic acid

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concentration and antioxidant activity when brown rice stored at different temperature (4°C and 37°C), and the relationship among these parameters.

MATERIALS AND METHODS

Rice samples

Rice grains were cultivated in the 2009-2010 growing season in Hei Long Jiang, China. After dehulled, two brown rice cultivarswereselected (both are long grains, named as Acheng/BAC and Wuchang/BWU) for this study. Bulk samples (3 kg) of two brown rice cultivars were stored at 4 and 37°C in sealed glass bottles for up to 6 months. During storage, the rice sample was withdrawn at different time interval, and ground using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO, USA) through a 0.5mm sieve screen. All the analysis was performed in triplicate.

Extraction of free phenolic compounds (Free-PC)

Before the extraction, rice sample was treated using pure hexane for twice times (1:20 g/v for each treatment) to remove lipids. After air-dried, the residue was used for extracting Free-PC using the modified methodology of Iqbal, Bhanger, and Anwar (2005) and Pérez-Jiménez and Saura-Calixto (2005). 1g of the residue was mixed with 20 mL of 80% methanol and homogenized for 2min. The mixture was magnetic stirred at room temperature for 1 h with the protection of light. The mixture was centrifuged at 2920 *g*for 10 min and the supernatant was collected. The residue was mixed with 10 mL of acetone/water/acetic acid (70/29.5/0.5, v/v/v) and repeated above procedure. 20 mL of 80% methanol (chilled, pH 2.0) was added to the collected residue for the third extraction. The three supernatants were combined together and used for the analysis of FPCs and antioxidant assay. The final residue was washed twice times using pure acetone, air-dried and then used for analysis of bound phenolic compound (Bound-PC).

Extraction of bound phenolic compounds (Bound-PC)

After the extraction of Free-PC, the dried residue was used for the determination of Bound-PC according to an alkaline extraction protocol in Zhou et al.[27]. Briefly, Rice flour (2.0 g, dry basis) was combined with aqueous NaOH (4M; 60 ml) and the mixture was stirred using a magnetic stirrer at room temperature under a nitrogen atmosphere for 4 h. The sample was acidified to pH 1.5–2.5 by gradual addition of ice-cold 6 M HCl and extracted three times with ethyl acetate (3×70 ml). The ethyl acetate fraction was dried by addition of anhydrous sodium sulfate and evaporated to dryness using a rotary vacuum evaporator at 35°C. The residue was redissolved in aqueous methanol (50% v/v; 4 ml), filtered through a 0.45μm nylon filter and stored in the dark prior to analysis by HPLC. The extractions were performed in triplicate.

Determination of total phenolicsconcentration

The total phenolics in both Free-PC andBound-PCwas analyzed using the Folin–Ciocalteu assay. Briefly, 0.1 mL of the extract (Free-PC and Bound-PC) was mixed with 0.25 mL of Folin-Ciocalteu reagent. After gentle shaking, 3 mL distilled, deionised water was added to the mixture. After incubation at room temperature for 2 min with the protection of light,1.0 mLof 15% sodium carbonate was added and left for 1 min, and then made volume to 10.0 mL with distilled, deionised water. After incubation at room temperature under dark for 90min, the absorbance was determined at 760 nm against the reagent blank. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolic content was expressed as mg gallic acid equivalent (GAE) per 100 g grains (mg GAE/100g).

Determination of total flavonoid concentration

The total flavonoid concentration of rice samples both in Free-PC and Bound-PC was determined by using a colorimetric method[21]. 1.00 mL of the extract was mixed with5 mL of distilled, deionized water in a 10 mL volumetric flask, then added 0.30 mL of 5% sodium nitrite. After 5 min, 0.30 mL of 10% $AlCl_3$ was added to the mixture.After 6 min, 2 mL of 1 $molL^{-1}$NaOH was added, followed by the addition of distilled, deionized water to the volume. The absorbance was measured immediately at 510 nm. The content of total flavonoids was calculated from the calibration curve of catechin standard. Measurements were calibrated to a standard curve of prepared catechin solution, and the

total flavonoid content was expressed as mg (+)catechin equivalent (CE) per 100 g grains.

Measurement of ferulicacid concentration in Free-PC and Bound-PC

The HPLC was applied for measuring ferulic acid concentration in the extracts (Free-PC and Bound-PC). An aliquot (250 μL) of the purified extract was separated using a Varian HPLC System (Model 9012, USA). Peaks were detected with a variable wavelength UV–vis detector (Varian 9050, USA) operated at 280 nm. Separations were achieved on a 5-lm Alltima C18 column (150 mm×4.6 mm; Alltech Associates, Inc, Australia). Gradient elution was performed with a gradient of A (water:acetic acid, 100:1, v/v) and B (methanol:acetonitrile:acetic acid, 95:5:1, v/v/v) as follows: 0–2 min, 5% B; 2–10 min, 5–25% B; 10–20 min, 25–40% B; 20–30 min, 40–50% B; 30–40 min, 50–100% B; 40–45 min, 100% B; 45–55 min, 100–5% B. Solvent flow rate was 1.0 ml min^{-1} and the temperature of the column was maintained at 22°C. Peak identities were confirmed from retention data and by spiking of extracts with authentic standards. Recovery of ferulic acid from spiked samplewas 81%. Quantification was achieved by reference to authentic compound used as external standard.

Determination of antioxidant activity (AOA)

In this assay, the antioxidant activity of the rice grain extracts (both Free-PC and Bound-PC) was assessed in terms of hydrogen donating or radical scavenging abilities using a method from Lopez-Martinez et al., 2009. Extracts were tested at a standardizedphenolic concentration of 0.1 mmol/L (gallic acid equivalents). Thereaction for scavenging DPPH radical was conducted in 15 mL polypropylene tubes (Becton–Dickinson) at room temperature (25°C). Extracts of rice tested at a phenolic concentration of 0.1 mmol/L (gallic acid equivalent) were added to 2.8 mL of 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^*$, 98.9 mmol/L in methanol) and vortexed for 15 s. The decrease in absorbance of $DPPH^*$ was measured at 515 nm in a photodiode array spectrophotometer, starting at the time the solution was added and then every hour until no further change in absorbance was measured (ca. 6 h). Methanol served as blank solution (equivalent methanol was added to test samples), Trolox (0.020 mmol/L) was a positive antioxidant control; and 2.8 mL of $DPPH^*$ plus 100 μL methanol served as a control. The AOA was calculated as a 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent (mmol TE) per g of grain by comparison to a standard curve. The reactions were performed in triplicate, and the mean value was obtained.

Statistical analysis

Experimental data were subjected to analysis of variance using Genstat 5 (release 4.1). Treatment means were tested separately for least significant difference (lsd) at a 5% level of probability.

RESULTS AND DISCUSSION

Phenolics profile of the two brown rice cultivars

The concentration of phenolic compounds present in the two rice cultivars and the corresponding antioxidant capacity were measured and the results were listed in TABLE 1. In general, cultivar BAC had a higher concentration of phenolic compounds (Free-PC and Bound-PC, respectively) than cultivar BWU, indicating the genetic influence on phenolic compounds of rice. Nevertheless, the data showed a similar phenolic profile between the two rice cultivars. For example, the concentration of Bound-PC was higher than that of Free-PC in the two tested rice samples. Adom and Liu[26] also reported that Bound-PCis a major portion of total phenolics (sum of free- and boundphenolics) in grains (85% in corn, 75% in oat and wheat, and 62% in rice). However, the report from Minet al.[4] showed anoppositephenolics profile after the measurement of 13 whole gran cultivars. The discrepancy might be due to the use of different extracting solvents: 80% chilled ethanol in Adom and Liu[1] and 100% methanol in Maillard and Berset[13], while acetone/water/acetic acid was used in Min et al. study.

The antioxidant activity of the two rice grains was evaluated and the results were seen in TABLE 1 and 2. The data showed that AOA for Free-PC extract were 3.2 and3.7 mmol TE per gram sample for BWU and BAC, respectively. This difference in the AOA between the two rice cultivars was highly consistent with their phenolic profile of Free-PC extract. This might suggest that rice BACmay serve better as a reducing agent than

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rice BWU. Flavonoids have been considered as strong iron chelating agents because the negatively charged phenoxide groups of deprotonated phenolic compounds chelate free ionic irons and stabilize the form of the free ionic irons[15]. It was also seen that, although theflavonoid concentration in Bound-PC was high than that in Free-PC (24.7*vs* 20.9 for rice BWU, 27.5 *vs* 21.2 for rice BAC), their corresponding AOAdemonstrated an opposite pattern. It has been known thatflavonoids are efficientreducing agentsand iron chelating agents. However, this study might suggest that, their reducing capacity, radical scavenging capacity, the iron chelating capacity of individual flavonoids may vary depending on their structure and the number of attached hydroxyl groups[15].

Effect of storage temperature on phenolic compounds in Free-PC extract

The effect of storage temperature on the phenolic compounds was summarized in Table 1. In general, the concentration of phenolics increased at the first 2 months storage, followed by a slight decrease but with no statistic difference for the two rice cultivars stored at 4°C. However, a consistent decrease in the concentration of phenolics and flavonoids was found following the storage at 37°C forboth cultivars. Other studies also have suggested that phenolic compounds may suffer decompositionunder high temperatures, and this effect depends on the temperature, time of processing, type of compounds inthe sample and other conditions[1,19]. This decomposition led to a reduction of the polyphe-

TABLE 1 : Concentration of free phenolic compounds (mg gallic acid equivalents per100g rice sample), free flavonoid compounds (mg (+)-catechin equivalents per 100g rice sample) and freeferulic acid, and antioxidant activity (AOA, mmol TE per g rice sample, dry base)

Free phenolic compounds														
Cultivar	**BWU**							**BAC**						
Storage temperature	**4℃**				**37°C**			**4℃**				**37℃**		
Storage time (month)	0	2	4	6	2	4	6	0	2	4	6	2	4	6
TPCs	43.5	44.3	42.7	42.5	42.1	40.2	37.3	45.1	45.9	45.0	45.2	42.4	40.3	39.1
TF	20.9	20.5	20.2	19.7	19.3	18.8	18.1	21.2	21.0	20.7	20.8	20.1	19.2	18.3
FER	17.2	17.9	17.0	16.5	16.3	15.2	14.4	17.9	18.4	17.8	17.5	16.8	16.0	15.2
AOA	3.2	3.1	3.0	3.0	2.8	2.2	1.7	3.7	3.5	3.5	3.4	3.1	2.6	2.1

TPC: total peholics; TF: total flavonoid; PER: ferulic acid; AOA: antioxidant activity.

TABLE 2 : Concentration of bound phenolic compounds (mg gallic acid equivalents per 100g rice sample), bound flavonoid compounds (mg (+)-catechin equivalents per 100g rice sample) and bound ferulic acid, and antioxidant activity (AOA, mmol TE per g rice sample, dry base).

Bound phenolic compounds														
Cultivar	**BWU**							**BAC**						
Storage temperature	**4℃**				**37°C**			**4℃**				**37℃**		
Storage time (month)	0	2	4	6	2	4	6	0	2	4	6	2	4	6
TPCs	67.3	68.3	66.7	66.5	68.1	67.2	65.3	69.3	69.9	68.6	68.2	68.5	67.3	67.0
TF	24.7	24.5	24.2	24.0	24.0	24.1	23.7	27.5	27.0	27.7	27.0	26.8	26.2	25.8
Ferulic acid	21.3	21.9	21.0	21.0	20.8	20.4	20.4	23.9	23.9	23.2	23.3	23.3	22.7	22.1
AOA	1.9	1.9	1.7	1.7	1.7	1.5	1.4	2.2	2.1	2.0	2.0	2.0	1.8	1.8

TPC: total peholics; TF: total flavonoid; PER: ferulic acid; AOA: antioxidant activity.

nol concentration, as observed in thepresent work for rice stored at 37°C.

Storage made great impact on AOAvalue (TABLE 1), depending on storagetemperature. The change in the AOA value during storage was consistent with the change in profile of phenolic compounds. Another plausible mechanism for the significant reduction in AOA for rice stored at 37°Cmight be the possibility of interaction of the phenolic compounds with other components in rice. One of the most outstanding chemical re-

actions occurred in rice during storage is lipid hydrolysis and oxidation. The fat acidity is commonly used as an index of qualitydeterioration during rice storage because lipid dissolutionprogresses more rapidly than that of protein and starch[17]. Genkawa, et al[7] have reported that the fat acidity was increased from 24.3 (fresh) to 59.1 and 68.8 mg KOH100 $g^{-1}$when brown rice was stored at 15°C and 25°C storage for 6 months, respectively. It is also important to make sure that the lipid oxidizable substrate does not initially contain oxidation compoundsor proxidant substances like transition metals. Because free fatty acids (FFA) are more susceptible to autoxidation than esterified fatty acids, the control of the concentration of the FFA, particularly unsaturated FFA might be an effective way for retarding rice ageing progress. Therefore, the existing of phenolic compounds in rice may be involvedin the regeneration of the chromanoxyl radical into chromanolthrough polyphenol oxidation into phenoxyl radicals.

Effect of storage temperature on phenolic compounds in Bound-PC extract

Similar to the changes in Free-PC, the concentration of phenolic compounds in Bound-PC extract also showed a reduction during rice storage at 37°C TABLE 2). This indicates that the chemical reactionsalso occurred in Bound-PC fractionduring rice ageing process. However, the magnitude of the reduction of Bound-PC after the storage was much lower than that of Free-PC, suggesting that the phenolic compounds in Free-PC extractwere more sensitive to ageing progress. This difference might be due to the different structures of phenolic compounds present in Free-PC and Bound-PC fractions, respectively, because structural difference is thedetermination of its individual reducing capacity of the phenolic compound. Many *in* vitro studies[5,6,8] have also shown that a 1,2-dihydroxy substitution on the B ring (catecholic structure) is a key factor in determining the antioxidant activity of phenolic compounds. The higher antioxidant efficacy of ortho-diphenols is usually explained by stabilization of the phenoxy AOradical through formation of an intramolecular hydrogen bond. In vivo, it is quite likely that flavonoids with a catechol-like B cycle act in the same way as ascorbic acid. Consistently,the rice stored at 37°C for 6 months displayed a greater reduction of 46.9 and 43.2% inAOA in Free-PC extract fraction compared to a reduction of 26.3 and 18.2% of AOA in Bound-PC extractfor the two rice cultivars. This further confirmed that phenolics existing in different fraction paly different roles on retarding rice ageingprogress, which might be based on their configuration structures.

The relationship between

The correlations among the parameters measured in this study are displayed in TABLE 3. The concentration of phenolics, flavonoid and ferulic acid in Free-PC fraction were highly correlated to the AOA value of their respective fractions (R^2ranges from 0.922 to 0.940). A relatively high correlation was also found among these parameters in Bound-PC (R^2 ranges from 0.922 to 0.940). This indicates that free and bound phenolics directly contribute to the antioxidant capacity of whole grain rice, but they might contribute to different influence on rice quality. It is assumed from this study that phenolic compounds in free fraction take main roles for controlling rice total AOA value, whereas phenolic compounds in bound fraction take partially contribution to rice AOA but with some other influence on rice quality during rice storage. For instance, the oxidation of ferulic acid in bound fraction led to form diferulic esters, which could make the cell wall structure of rice more strengthened, and subsequently impact rice cooking behaviors.

TABLE 3 : Correlation coefficients between phenolics and antioxidant activity measured in this study

	Free fraction			Bound fraction		
Phenolic compounds	TPCs	TF	Ferulic acid	TPCs	TF	Ferulic acid
AOA	0.940	0.936	0.922	0.749	0.707	0.828

TPC: total peholics; TF: total flavonoid; PER: ferulic acid; AOA: antioxidant activity

CONCLUSION

Although the concentration of Bound-PC was higher than that of Free-PC in the two tested rice samples, their corresponding AOA demonstrated an opposite pattern. This study might suggest that the structure and profile of phenolic compounds may differ between the two fractions. There was a significant reduction in the

concentration of phenolic compounds and AOA both in Free-PC and Bound-PC fractions following the rice storage at 37°C, indicating phenolic compounds present in the two fractions are all involved in the chemical reactions occurred during rice ageing process.

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