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ARTICLE

Changes in Phenolic Acids and Antioxidant Activity in Thai Rice Husk at Five Growth Stages during Grain Development

SUNAN BUTSAT,[†] NATTHIDA WEERAPREEYAKUL,[‡] AND SIRITHON SIRIAMORNPUN*,[†]

[†]Department of Food Technology and Nutrition, Mahasarakham University, Mahasarakham 44000, Thailand, and [‡]Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

Soluble and bound phenolic acids were isolated from Thai rice husk samples at five growth stages during grain development, and their antioxidant activities were evaluated. The results showed that ferulic acid was the major soluble phenolic acid in husk at all stages, and its concentration decreased steadily during grain development. The ratio of ferulic to *p*-coumaric acid was approximately 2:1 at all stages. The most abundant bound phenolic acid in all extracts was *p*-coumaric acid, followed by ferulic acid along with traces of syringic, vanilic, and *p*-hydroxybenzoic acids. Most of the antioxidant activities of soluble and bound phenolic acids in husk extracts were found at flowering stage, and there were high correlations of antioxidant activity to levels of soluble ferulic, gallic, and *p*-coumaric acids.

KEYWORDS: Ferulic acid; *p*-coumaric acid; rice husk; ferric reducing ability; growth stages

INTRODUCTION

Generally, the outer layers of plants, such as the peel, shell, and hull, contain large amounts of polyphenolic compounds to protect the inner materials. Several hundreds of different plant polyphenols have been identified (*1*). Phenolic acids are the predominant phenolic compounds found in brown rice, especially ferulic acid and *p*-coumaric acid (*2*). They exist in three forms, namely, free, soluble conjugated, and insoluble bound; this last form is found in dietary fiber (*3*). Currently, agricultural and industrial residues are being studied as attractive sources of natural antioxidants (*4*). Rice husk, a prominent example of this type of residue, is removed during the rice milling process; it is considered a waste byproduct, with little economic value. However, rice husks contain an antioxidant defense system that protects rice seed from oxidative stress (*5*). In particular, isovitexin isolated from rice husk has been shown to exert strong antioxidant activity by inhibiting lipid peroxidation (*6*). Jeon et al. (*7*) reported that phenolic compounds from methanolic extracts of rice husk exhibited high antioxidant activity against scavengers of singlet oxygen and inhibited hydrogen peroxide-induced damage to cellular DNA in human lymphocytes. Recently, Kim et al. (*8*) identified momilactone B from rice husks as the active compound having cytotoxic and antitumor activity against human colon cancer cells. As a result, rice husks are considered an economically attractive source of natural antioxidants, leading to many attempts to elucidate their biochemical mechanisms in protection against oxidative stress-induced damage. However, information about the changes in phenolic acid composition of husk during grain development is scarce.

In this study, we aimed to investigate the changes in phenolic acid content in rice husk of Khao Dawk Mali 105 rice, the most famous exported Thai rice which has not been previously reported. It is nonpigmented rice and a fragrant as well as good quality cooked rice. We also further explored the changes in phenolic acid composition and content in rice husk during grain development and evaluated the radical scavenging properties and total phenolic contents. This study was designed to improve our understanding of the antioxidant properties of rice husk during grain development. This information could be potentially useful if rice husk is to be upgraded from its present status of having no significant use except for fuel.

*To whom correspondence should be addressed. Telephone: +66 857 474136. Fax: +66 43743135. E-mail: sirithons@hotmail.com.

MATERIALS AND METHODS

Samples. Rice husk from the rice cultivar *Oryza sativa* L. Indica was collected from Mahasarakham, in the northeastern region of Thailand. Approximately 500 g of paddy grains was collected 7, 14, 21, 28, and 35 days after flowering (DAF), corresponding to the flowering, milk grain, dough grain, maturity, and fully ripe grain stages, respectively, as described in **Table 1**. The husks were removed and separated from grains manually, then lyophilized, ground in a mill, and passed through a 35-mesh sieve.

Chemicals. The phenolic acid standards, such as gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, ferulic, *p*-coumaric, caffeic, chlorogenic, and sinapic acids, were purchased from Sigma Chemical Co. (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromancarboxylic acid (Trolox), and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Fluka. Other chemicals and solvents were of analytical grade.

Extraction of Soluble Phenolic Compounds. Soluble phenolic compounds refer to free and conjugated phenolic compounds in rice husk (1 g) and were extracted with 80% methanol (3 × 10 mL, 1 h each) by shaking at 150 rpm in an incubator at 37 °C. Each extract was pooled and evaporated at 45 °C to 10 mL under reduced pressure and was then lyophilized to dryness; dry weight was recorded and yield calculated. Crude extracts were dissolved in 5 mL of methanol and subjected to HPLC analysis. All analyses were performed in triplicate.

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Table 1. Description of the Five Growth Stages of Grain Development Used in This Study

growth stage	days after flowering (DAF)	description
flowering	0−7	One or more florets on the main stem panicle have reached anthesis until at least one caryopsis on the main stem panicle is elongated to the end of the husk. The grain husks are green.
milk grain	8−14	The endosperm first begins to form as a milky liquid. At least one caryopsis on the main stem panicle has a caryopsis filling the cavity of the lemma and palea (the husk). The grain husks are green.
dough grain	15−21	The milky liquid begins to solidify into a sticky white mass. At least one grain on the main stem panicle has a yellow husk.
maturity	22−28	The grain is mature, or ripe, when the endosperm becomes hard and opaque. At least one grain on the main stem panicle has a brown husk.
fully ripe	29−35	All grains have a brown husk.

Extraction of Bound Phenolic Compounds. The bound phenolic contents were extracted according to the method of Adom et al. (*9*) with minor modifications. Briefly, the residue from soluble fractions described above were drained off and hydrolyzed directly with 2 M sodium hydroxide at room temperature for 1 h with shaking under nitrogen gas, and the solution was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were dissolved with 5 mL of methanol and analyzed by HPLC. All analyses were performed in triplicate.

Determination of Total Phenolic Content (TPC). The TPC of rice husk extracts was determined using the method of Adom et al. (*9*) with some modifications. Briefly, 0.2 mL of rice husk extract was mixed with diluted Folin-Ciocalteu reagent and allowed to react for 5 min. Then, 0.8 mL of a 10% Na_2CO_3 solution (w/v) was added, and the final volume was increased to 5 mL with deionized water. After reaction for 2 h at room temperature, the absorbance at 760 nm was measured. Using gallic acid as a standard, TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of husk.

DPPH Radical Scavenging Activity. The free radical scavenging activity of husk extracts was determined according to the method of Liu et al. (*10*) with minor modifications. Rice husk extracts (0.1 mL) were added to 1.9 mL of a 0.1 mM methanolic solution of DPPH. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 30 min. Inhibition of free radical DPPH in percent (I%) was calculated by using the formula $I\% = [(A_0 - A_e)/A_0] \times 100$, where A_0 is the absorbance of the blank sample and A_e is the absorbance of the tested extract.

ABTS Radical Cation Scavenging Activity. The radical scavenging activity of rice husk extracts against ABTS radical cation was measured using the method of Zhao et al. (*11*) with some modifications. ABTS was dissolved in water to a concentration of 7 mM. ABTS radical cations were produced by reacting an ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. The $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. An aliquot of each rice husk extract (0.15 mL) was mixed with 2.85 mL of a dilute $ABTS^{\bullet+}$ solution. After reaction at 30 °C for 20 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of $ABTS^{\bullet+}$ scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry rice husk.

Ferric Reducing Ability of Plasma Assay (FRAP). The FRAP assay is a method of measuring the antioxidants to reduce Fe^{3+} and Fe^{2+}. The formation of the blue-colored Fe^{2+}−TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. This was described by Benzie et al. (*12*). Freshly prepared FRAP reagent [1.7 mL, including acetate buffer (pH 3.6), TPTZ, and $FeCl_3$] was mixed with 0.3 mL of sample extract. The absorbance at 593 nm of the mixture was measured after reaction for 60 min. The reducing power was calculated from the standard curve constructed using an $FeSO_4$ solution.

Determination of Phenolic Acid Composition. For high-performance liquid chromatography (HPLC) analysis, each residue was dissolved in 5 mL of methanol (HPLC) and then passed through a 0.45 μm filter. A 20 μL aliquot of sample solution was fractionated using a Shimadzu HPLC system equipped with a diode array detector on a 250 mm × 4.6 mm (inside diameter), 5 μm, Inertsil C18 analytical column. The mobile phase consisted of purified water with phosphoric acid (pH 2.58) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; from 22 to 38 min, linear gradient from 11 to 18% solvent B; from 38 to 43 min, from 18 to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. The column temperature was set at 38 °C. Hydroxybenzoic acid compounds were detected at a wavelength of 280 nm and hydroxycinnamic acid compounds at 325 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

Statistical Analysis. Experimental results are given as means ± the standard deviation of three parallel measurements. Analysis of variance and least significant difference tests were conducted to identify differences among means, while a Pearson correlation test was conducted to determine the correlations among means. p values of < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Extraction Yields of Soluble Phenolic Acids. The extraction yield of soluble phenolic acids refers to free and conjugated phenolic acids extracted with 80% methanol, whereas that of bound phenolic acid refers to alkaline-hydrolyzed extract expressed as the dry weight of crude solid material extracted per gram of dry husk at five growth stages; values are presented in **Table 2**. The extraction yields of soluble extracts showed significant ($p < 0.05$) differences among growth stages. The yields decreased progressively from flowering (8.3 g/100 g), followed by milk grain (5.1 g/100 g) and dough grain (3.5 g/100 g), to mature (2.4 g/100 g) and then increased slightly again at the fully ripe stage (2.5 g/100 g). The decrease in extraction yield during grain development probably occurred because soluble phenolics were progressively metabolized into lignin (*13*). This result agrees with the yields from *Gevuina avellana* hulls (*14*) and oat hulls (*15*). In the case of bound extracts, the flowering stage also showed the greatest extraction yield (2.7 g/100 g) and the lowest in extract at the fully ripe stage (2.3 g/100 g). However, there were no significant differences among other bound extracts.

Total Phenolic Content (TPC) of Rice Husk. Phenolic compounds may contribute directly to antioxidant action (*16*); therefore, it is necessary to investigate TPC. The TPC values expressed as milligrams of gallic acid equivalents (GAE) per gram of husk are listed in **Table 2**. The highest content of the soluble TPC in husk extract was observed at the flowering stage (2.1 mg of GAE/g). Later, during grain development from milk grain to maturity, the level of soluble TPC decreased slightly, ranging from 1.7 to 1.1 mg of GAE/g, and then increased at full ripeness. Our results are consistent with a previous report about other cereals (*17*)

Table 2. Extraction Yield and Total Phenolic Content of Rice Husk Extracts at Different Growth Stages during Grain Development

	extraction yield of dry weight[a] (g/100 g of husk)		total phenolic content (TPC)[a] (mg of GAE/g of husk)	
growth stage	soluble	bound	soluble	bound
flowering	8.3 ± 0.6 d	2.7 ± 0.1 b	2.1 ± 0.0 d	8.0 ± 0.3 b
milk grain	5.1 ± 0.2 c	2.5 ± 0.4 ab	1.7 ± 0.0 c	7.2 ± 0.7 ab
dough grain	3.5 ± 0.0 b	2.4 ± 0.0 a	1.6 ± 0.1 c	7.4 ± 0.4 ab
maturity	2.4 ± 0.0 a	2.4 ± 0.0 a	1.1 ± 0.1 a	6.6 ± 0.5 a
fully ripe	2.5 ± 0.0 a	2.3 ± 0.1 a	1.3 ± 0.0 b	7.1 ± 0.4 a

[a] Means followed by different letters within columns are significantly different ($p < 0.05$).

which reveals that rye caryopses at an early stage of development usually contained larger amounts of the soluble TPC compared to other stages. Similarly, bound TPC of husk extracts was the highest at the flowering stage (8.0 mg of GAE/g). The lowest bound TPC was found in husk extracts at maturity stage (6.6 mg of GAE/g), but there were only minor differences in bound TPC for husk extracts at earlier stages. On average, bound TPC was ~3−6 times higher than soluble TPC in all stages. This finding was in agreement with the results of Weidner et al. (*17*) that the content of phenolic acids liberated from soluble esters in developing rye caryopses was generally much higher than the level of free phenolic acids. Other previous studies reported on hydroxycinnamic acids in cereal straw (*18*) and sugar cane bagasse (*19*), in which an increase in the level of alkaline treatment had a significant effect on the release of ester-linked phenolic acids from the cell walls of cereal straw and sugar cane bagasse.

Changes in Phenolic Acids. Phenolic acids can be classified as free, soluble conjugated, and bound phenolic acids (*20*, *21*). Bound phenolic acids are typically involved in cell wall structure (*18*, *22*) where the cross-linking esters of lignin components via phenolic acids appear to have a profound effect on the growth of the cell wall and its mechanical properties and biodegradability. The HPLC chromatogram of standard phenolic acids and soluble extract of rice husk is shown in **Figure 1**. The contents of individual phenolic acids in husk extracts from different growth stages during grain development are summarized in **Tables 3** and **4**.

Soluble Phenolic Acids. For all growth stages during grain development, the total of individual soluble phenolic acids varied considerably, ranging from 76.8 μg/g of husk at maturity to 263.4 μg/g of husk at the flowering stage (**Table 3**). The results indicated that *p*-hydroxybenzoic, ferulic, and *p*-coumaric acids were the major soluble phenolic acids in the husk, accounting for 42, 24, and 12% of the total phenolic acids, respectively, at flowering. Minor constituents were gallic, protocatechuic, vanillic, chlorogenic, and syringic acids. The contents of ferulic acid were found to be major phenolic acids compared to other phenolic acids at all stages, with the exception of flowering. The contents of *p*-hydroxybenzoic and vanilic acids decreased from flowering (110.4 and 10.7 μg/g, respectively) to maturity (10.8 and 7.8 μg/g, respectively) and then again increased slightly at the fully ripe stage. On the other hand, the amount of ferulic acid steadily decreased from 64.2 μg/g at flowering to 18.1 μg/g at fully ripe. Levels of the other soluble phenolic acids fluctuated during grain development.

Bound Phenolic Acids. The contents of bound phenolic acids, bound to polysaccharides or lignins in the husk cell walls, are listed in **Table 4**. With respect to variation of bound phenolic acid content in all extracts, the flowering stage exhibited the highest total bound phenolic acids (10209 μg/g). The most abundant bound phenolic acid in all extracts was *p*-coumaric acid, followed by ferulic acid along with traces of syringic, vanilic, and *p*-hydroxybenzoic acids. Compared to soluble phenolic acids,

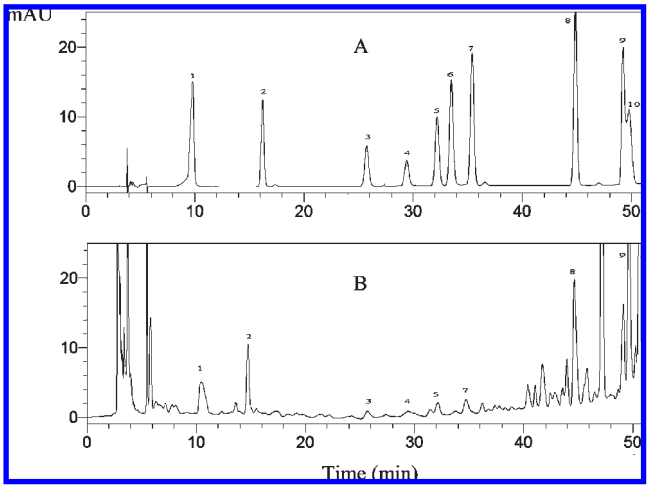

Figure 1. HPLC chromatogram of standard phenolic acids (**A**) and husk extract (**B**): (1) gallic acid, (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (4) chlorogenic acid, (5) vanillic acid, (6) caffeic acid, (7) syringic acid, (8) *p*-coumaric acid, (9) ferulic acid, and (10) sinapic acid.

the contents of bound phenolics were 250-fold greater for *p*-coumaric acid and 30−100-fold greater for ferulic acid. These results agree with previous studies of other grains, such as wild rice (*22*) and rice (*23*). Bound phenolic acids in the cell wall, particularly ferulic and *p*-coumaric acids, are known to be ester-linked to various cell wall polymers, namely, polysaccharides and the lignin component (*24*). Similar results have been reported by Iiyama et al. (*25*) and Lozovaya et al. (*26*). These authors stated that the walls of graminaceous monocots typically contain larger amounts of hydroxycinnamic acids than dicotyledons and *p*-coumaric acid tends to be the main hydroxycinnamic acid in the stems and stalks of cereals, whereas ferulic acid is enriched in the cereal bran. In this study, ferulic acid was the main compound among the soluble phenolic acids, whereas *p*-coumaric acid was the dominant compound in the bound form. The phenolics detected were those that are esterified to cell wall hemicellulosic and lignin polymers. Hence, it is very likely that some phenolic esters are liberated during the saponification in alkaline solution. Nevertheless, a considerable proportion of phenolic acids were identified. The results are listed in **Table 4**. As expected, the dominant phenolic acids were *p*-coumaric acid (5057−8027 μg/g) and ferulic acid (1825−1983 μg/g). Small amounts of other phenolics, such as syringic acid (247.2−310.5 μg/g) and vanillic acid (24.4−155.9 μg/g), were also found.

In summary, rice husk extracts in all stages of grain development were dominated by ferulic and *p*-coumaric acids. The contents of soluble phenolics were very low, whereas the vast majority was esterified to the cell wall (bound phenolics).

DPPH Radical Scavenging Activity. The DPPH radical is stable and is widely used to evaluate the radical scavenging activity of antioxidant compounds. The ability to act as donors of hydrogen

Table 3. Soluble Phenolic Acid Contents[a] (micrograms per gram) of Husk Extracts at Different Growth Stages during Grain Development

growth stage	gallic acid	protocatechuic acid	*p*-hydroxybenzoic acid	chlorogenic acid	vanillic acid	syringic acid	*p*-coumaric acid	ferulic acid	total (μg/g)
flowering	9.9 ± 1.7 b	24.0 ± 1.4 c	110.4 ± 2.9 d	8.3 ± 0.6 c	10.7 ± 0.8 b	3.3 ± 0.5 a	32.5 ± 1.1 d	64.2 ± 1.3 c	263.4
milk grain	5.4 ± 0.1 a	10.0 ± 0.7 ab	24.7 ± 1.4 c	5.4 ± 1.3 ab	9.3 ± 0.1 ab	2.6 ± 0.3 a	18.1 ± 0.0 ab	59.6 ± 2.8 c	135.0
dough grain	7.8 ± 0.1 ab	13.1 ± 1.0 b	19.6 ± 1.7 bc	11.3 ± 0.4 d	7.9 ± 0.2 a	8.6 ± 1.1 b	23.6 ± 1.1 c	46.2 ± 0.5 b	138.1
maturity	5.8 ± 0.3 a	6.7 ± 1.1 a	10.8 ± 0.7 a	8.0 ± 0.6 bc	7.8 ± 0.1 a	4.6 ± 0.3 a	14.8 ± 0.2 a	18.3 ± 0.6 a	76.8
fully ripe	7.1 ± 0.2 ab	10.4 ± 0.4 ab	13.0 ± 1.7 ab	4.8 ± 0.1 a	14.1 ± 0.2 c	12.1 ± 0.4 c	19.8 ± 1.2 b	18.1 ± 0.1 a	99.4

[a] Each value is the mean ± the standard deviation (n = 3). Means with different letters in the columns for each extract are significantly different ($p < 0.05$).

Table 4. Bound Phenolic Acid Contents[a] (micrograms per gram) of Husk Extracts at Different Growth Stages during Grain Development

growth stage	gallic acid	protocatechuic acid	*p*-hydroxybenzoic acid	chlorogenic acid	vanillic acid	syringic acid	*p*-coumaric acid	ferulic acid	total (μg/g)
flowering	ND[b]	ND[b]	16.6 ± 0.6 b	ND[b]	24.4 ± 1.5 a	247.2 ± 5.6 a	8027 ± 245 c	1894 ± 109 a	10209
milk grain	ND[b]	ND[b]	8.4 ± 1.1 a	ND[b]	61.8 ± 1.7 b	310.5 ± 17.9 b	5057 ± 172 a	1906 ± 80 a	7343
dough grain	ND[b]	ND[b]	31.4 ± 2.7 d	ND[b]	107.5 ± 1.9 c	298.7 ± 6.2 ab	6250 ± 95 b	1983 ± 109 a	8670
maturity	ND[b]	ND[b]	24.2 ± 0.4 c	ND[b]	155.9 ± 9.9 d	294.4 ± 16.4 ab	5380 ± 86 a	1897 ± 114 a	7751
fully ripe	ND[b]	ND[b]	52.6 ± 2.3 e	ND[b]	118.5 ± 5.4 c	303.2 ± 12.6 b	5917 ± 56 b	1825 ± 95 a	8216

[a] Each value is the mean ± the standard deviation (n = 3). Means with different letters in the columns for each extract are significantly different ($p < 0.05$). [b] Not detected.

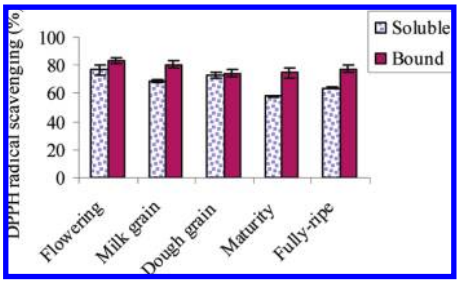

Figure 2. DPPH radical scavenging activity (percent) of rice husk extracts at five growth stages during grain development. Results are presented as means ± the standard deviation for triplicate analyses.

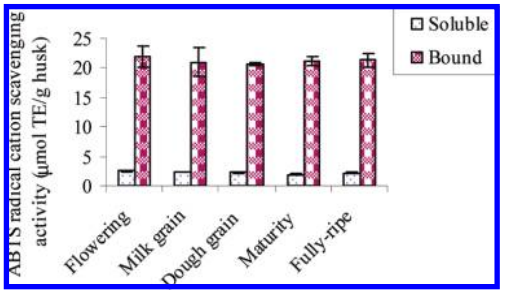

Figure 3. ABTS radical cation scavenging (micromoles of TE per gram of husk) of rice husk extracts at five growth stages during grain development. Results are presented as means ± the standard deviation for triplicate analyses.

atoms in the transformation of the DPPH radical to its reduced form (DPPH$^{\bullet}$-H) was investigated for rice husk extracts. The DPPH scavenging activity of husk extracts was reported as the percent inhibition of DPPH radical, with a higher value of percent of inhibition associated with a stronger antioxidant activity. The radical scavenging ability of all extracts is shown in **Figure 2**. All husk extracts, in the soluble form, exhibited moderately active inhibition of the scavenging DPPH radical. Among the five growth stages studied, the husk extract at the flowering stage showed the highest antioxidant activity (76.6%), followed by the dough grain (72.9%), milk grain (68.7%), fully ripe grain (63.9%), and maturity stages (58.1%). The large amount of total phenolics contained in husks at the flowering stage may account for its strong antioxidant activity. Several studies exhibited good correlation between total phenolic content and antioxidant activity of plant extracts (*27*), whereas the bound extracts exhibited radical scavenging activities slightly higher than those of the soluble extracts at all stages. In addition, the bound extracts at flowering and milk grain stages exhibited radical scavenging activities significantly ($p < 0.05$) higher than those of any of the other stages. Overall, results showed that all rice husk extracts were found to have potent antioxidant activity.

ABTS Radical Cation Scavenging Activity. The ABTS radical scavenging test is widely used to determine the antioxidant activity of both hydrophilic and lipophilic compounds. The estimation of antioxidant activities of plant phenolics is based on the inhibition of ABTS$^{\bullet+}$. **Figure 3** shows the relationship among the antioxidant activity of extracts, expressed as micromoles of Trolox equivalents (TE) per gram of husk. The results show that the antioxidant capacity of all the extracts decreased during grain development. For example, at the flowering stage, the antioxidant capacity of the extract decreased from 2.5 to 1.9 μmol of TE/g of husk at maturity stage, increasing again at fully ripe stage, whereas there were negligible differences among the bound extracts. However, the husk extracts of the bound form exhibited 10-fold higher antioxidant capacity for the ABTS assay compared to the soluble forms in all stages. The decreases in antioxidant capacity might be caused by the decreases in the levels of phenolics. The correlation coefficients between TPC and ABTS assay and between TPC and total soluble phenolic acid were 0.946 and 0.947 ($p < 0.05$), respectively (data not shown).

Ferric Reducing Ability Power (FRAP). The antioxidant capacity of rice husk extracts was also evaluated by the FRAP assay as shown in **Figure 4**. For soluble extracts during grain development, the extracts at milk grain and dough grain stages had significantly ($p < 0.05$) higher FRAP values than the other stages, whereas the extract at the mature stage had the lowest FRAP value (11.7 μmol of $FeSO_4$/g of husk). For bound extracts, the reducing power of antioxidant capacity decreased from the flowering stage (62.1 μmol of $FeSO_4$/g of husk) to maturity (51.8 μmol of $FeSO_4$/g of husk) and then again increased slightly at the fully ripe stage (53.3 μmol of $FeSO_4$/g of husk).

Correlations among Antioxidant Activity and Some Phenolic Acid and Total Phenolic Contents. All extracts of husk at different growth stages were used to analyze the correlations among free radical scavenging activities, based on DPPH and ABTS assays, ferric reducing ability, and individual and total phenolic contents. **Table 5** showed that in soluble extracts, the gallic acid content gave strong positive correlations with DPPH and ABTS$^{\bullet+}$

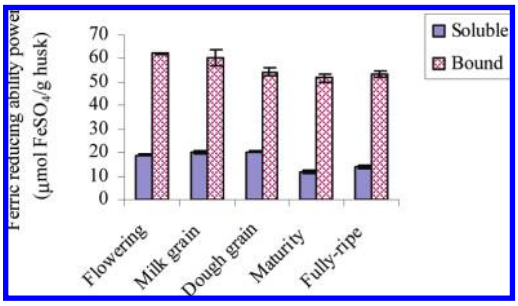

Figure 4. Ferric reducing ability (micromoles of $FeSO_4$ per gram of husk) of rice husk extracts at five growth stages during grain development. Results are presented as means ± the standard deviation for triplicate analyses.

Table 5. Correlations[a] among Rice Husk Antioxidant Activities and Some Phenolic Acid and Total Phenolic Contents

	soluble			bound		
	GA	FA	*p*-CA	FA	*p*-CA	VA
TPC	0.980[b]	0.943[c]	0.860	0.464	0.837	−0.907[c]
DSA	0.923[c]	0.863	0.887[c]	0.100	0.540	0.700
ASA	0.948[c]	0.888[c]	0.758	0.358	0.632	0.488
FRAP	0.707	0.889[c]	0.539	0.191	0.505	−0.979[b]

[a] Abbreviations: TPC, total phenolic contents; DSA, DPPH radical scavenging activity; ASA, ABTS radical cation scavenging activity; FRAP, ferric reducing ability power; GA, gallic acid; FA, ferulic acid; *p*-CA, *p*-coumaric acid; VA, vanillic acid. [b] Correlation is significant at the 0.01 level (two-tailed). [c] Correlation is significant at the 0.05 level (two-tailed).

scavenging activities, while the ferulic acid content exhibited strong positive correlations with $ABTS^{\bullet+}$ scavenging and FRAP assays. In addition, the content of *p*-coumaric acid showed a high correlation with the DPPH assay ($r = 0.887$; $p < 0.05$). Our results showed that the soluble extracts had a high correlation among total TPC and radical scavenging activities, including DPPH, ABTS, and FRAP with correlation coefficients of 0.935, 0.946, and 0.797, respectively (data not shown), which is consistent with results reported by Zielinski et al. (*28*) and Adom et al. (*9*) concerning phenolics and antioxidant activity of grains. On the other hand, for bound extracts, vanillic acid content showed negative correlations with total phenolic content ($r = -0.907$; $p < 0.05$) and FRAP value ($r = -0.979$; $p < 0.01$). Finally, it should be pointed out that the different antioxidant responses and levels of phenolic acids at various growth stages can probably be interpreted in light of correlations among husk antioxidant activities.

Conclusions. The phenolic acid profiles of rice husks during grain development were monitored from the flowering stage to the fully ripe stage. The highest levels of these compounds were found at flowering and the lowest level of maturity. Our results revealed that ferulic acid was the major soluble phenolic acid found in all husk extracts during the development stages, accounting for 18−44% of total phenolic acids. On the other hand, *p*-coumaric was the main bound phenolic acid of husk (approximately 70−78% of total phenolic acids), and ferulic acid was a more minor constituent present in the bound form (18−25% of total phenolic acids). Our results also showed the major portions of phenolic acids in the husk were present in the bound form, presumably attached to cell wall material. Most of the antioxidant activity was found at the flowering stage, and there were high correlations of antioxidant activity to levels of soluble ferulic, gallic, and *p*-coumaric acids. Our results demonstrate that the levels and composition of the phenolic acids change considerably during the growth and development of rice grain. The phenolic acid and antioxidant properties of husk extracts indicate that rice husk may be regarded as a valuable source of an antioxidant-rich nutraceutical. The potential of applications of rice husk antioxidants could be in value-added processed foods or the cosmetic industry.

ABBREVIATIONS USED

DAF, days after flowering; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromancarboxylic acid; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; FRAP, ferric reducing ability power; TPC, total phenolic content; GAE, gallic acid equivalents; TE, Trolox equivalents; DSA, DPPH radical scavenging activity; ASA, ABTS radical cation scavenging activity; HPLC, high-performance liquid chromatography.

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