Bioactive compounds and antioxidant properties of different solvent extracts derived from Thai rice by-products

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Abstract We investigated the contents and compositions of bioactive compounds in by-products of rice as affected by extraction with different solvents. Free and bound phenolic compounds and their antioxidant activities of rice bran and husk extracted by acetone, ethanol, and water at different temperatures (50, 60, 70, and 80 °C) were evaluated. Overall, the heated water extract provided the highest 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities, ferric-reducing antioxidant power, total phenolic content, and total flavonoid content compared to the other solvents of all the samples studied. The antioxidant activities increased when the water temperature increased from 50 to 70 °C but decreased at 80 °C. The contents of bound phenolics were greater than those of free phenolics, including phenolic acids and flavonoids, in all the samples studied. Acetone gave the highest amounts of γ-oryzanol and tocopherols in all samples. With a reduction in particle size of the rice husk, there was a significant increase in extracted phenolic acids, flavonoids, and antioxidant properties.

Keywords Acetone · Bound phenolics · Hydrocinnamic acid · Myricetin · Rice husk

Introduction

Rice is a staple food in many regions of the world, especially in Asian countries. Approximately 21–26 million tons of rice is produced annually around the world (OAE 2003). In Thailand rice is the most important crop in terms of production, consumption, and export. A large amount of rice by-products derived from the milling process, included husks, bran, and a little amount of broken rice, are produced (Onyeneho and Hettiarachchy 1992), and they have been underutilized, although a small proportion of the rice bran is used for making rice bran oil. A number of studies have reported the presence of bioactive compounds in these rice fractions. For instance, rice bran is a rich source of oryzanols or sterolaryl esters (Seetharamaiah and Prabhakar 1986; Norton 1995). In addition, rice bran could be a potential source of tocopherols, tocotrienolsIn general phenolic compounds (Nicolosi et al. 1994), which have shown great antioxidant activity (Xu et al. 2001; Nam et al. 2005). Furthermore, rice husks offer the beneficial nutritional advantage that they contain an antioxidant-defense system to prevent the rice seed from being exposed to oxidative stress (Ramarathnam et al. 1988). Rice husk has been reported to contain a large amount of phenolic compounds (Butsat and Siriamornpun 2010). However, an appropriate extraction or isolation method of the bioactive components from rice bran and rice husk is needed to achieve the most effective means for obtaining the greatest amount and biological activities.

Factors that influence the yield and health promoting properties of the bioactive compounds include the nature of the material matrix, extraction techniques, and extracting...
solvents (Al-Farsi and Lee 2008). It has been known that the antioxidant properties mostly depend on the nature of the solvents, as different antioxidants and bioactive compounds respond differently toward the solvent (Antolovich et al. 2000; Karabegovic et al. 2014). The presence of various antioxidant compounds with different chemical properties may or may not be soluble in a specific solvent (Turkmen et al. 2006). Polar solvents are often used for recovering phenolic compounds from plant matrices. In general, for the extraction of phenolic compounds or various bioactive compounds from plant materials, water, and organic solvents (ethanol, methanol, acetone and diethyl ether) are used. Ethanol is known to be a good solvent for phenolic compound extraction, and it is safe for human consumption, while acetone is a good solvent for extracting non-polar compounds like aglycone flavonoids, but it is toxic (Dai and Mumper 2010). Water is the safest and also most environmentally friendly and easily obtainable solvent (Vuong et al. 2011). It is also significantly less expensive than organic solvents, which have been traditionally used for plant bioactive extractions. Theoretically, the best extraction method should be simple, safe, reproducible, cheap and suitable for industrial application (Vongsak et al. 2013). The contents of bioactive compounds are affected by the method of extraction, which indicates that the extraction solvents used and their polarity should be considered when needing to increase the bioactive markers as well as enhanced bioactivities (Sun and Ho 2005; Turkmen et al. 2006; Hayouni et al. 2007; Luthria 2012). Up to date, there has been no information available in the literature on the use of different extraction solvents to increase the contents of bioactive compounds, especially phenolic compounds and antioxidant activities from Thai rice by-products. Thus, it was of our interests to investigate the effects of the effect of various extraction solvents, namely acetone, ethanol and water at different temperatures (50, 60, 70, and 80 °C) on bioactive compounds, including phenolic acids, flavonoids, γ-oryzanol, and tocopherols along with the antioxidant activities of Thai rice by-products (rice bran and husk). We hope to provide an appropriate method for the extraction of bioactive components from rice bran and rice husk.

**Materials and methods**

**Sample preparation**

Paddy-rice samples (KDML 105 variety) were harvested from Mahasrakaham Province, northeastern Thailand. These grains were milled to separate the husks from the brown rice. Then the brown rice was polished to obtain the bran. The husks were ground and passed through a 500 μm sieve screen, and this was called the ground rice husk. The moisture was determined by drying at 110 °C to constant mass. This and all other analyses were performed using triplicate samples, and analytic results are expressed on a dry matter basis. The samples were stored at −20 °C prior to analysis.

**Chemicals and reagents**

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH*), 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu’s reagent and standards of phenolic compounds (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, rutin, myricetin, quercetin, apigenin, and kaempferol) were obtained from Fluka (Germany). Oryzanol (food grade, 99.9 % purity) was obtained from Tsuno Rice Fine Chemicals Co., Ltd. (Japan). The acetic acid, methanol, acetonitrile and other solvents, and reagents used in the high-performance liquid chromatography (HPLC) analysis were purchased from Merck (Germany). All chemicals and reagents used in the study were of analytical grade.

**Extraction preparation for antioxidant activity determination**

Three solvents, including ethanol, acetone and water were selected for using in antioxidant compounds extraction by their polarity as described by Kubola et al. (2013). Non-polar or less polar compounds such as aglycone flavonoids may be extracted by acetone, while ethanol was used to extracted medium polar compounds like phenolics, flavonoids, and glycosides. Polar compounds such as glucosides and sugar could be dissolved in water. For the extractions with absolute ethanol and acetone, 1 g of each sample was extracted with 10 mL of each solvent for 2 h at room temperature. The water extraction was performed at four different temperatures (50, 60, 70, and 80 °C) in a thermostated water-bath (UMAX, UM-SW 50L). The mixtures were then filtered through filter paper (Whatman No. 1, Sigma–Aldrich Co., USA), and the filtrate was used for analyzing the antioxidant activity in vitro.

**DPPH radical scavenging activity**

The DPPH scavenging activity of the extracts was measured as described by Butsat and Siriamornpun (2010) with some modifications. In brief, the sample extract (0.1 mL) was mixed with 1.9 mL of 0.1 mM DPPH in ethanol. The mixture was vortexed (1 min), left to stand at room temperature in the dark (30 min) and then the absorbance of the solution was read at 517 nm. The percent inhibition activity was calculated as \[I = \left(\frac{A_o - A_e}{A_o}\right) \times 100\] (\(A_o\) = absorbance without extract; \(A_e\) = absorbance with extract).
**Ferric reducing/antioxidant power (FRAP) assay**

The FRAP assay was conducted as described by Benzie and Strain (1996). The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl) and 10 mL FeCl₃·6H₂O (20 mM) in a ratio of 10:1:1 and 12 mL of distilled water, at 37 °C. To perform the assay, 1.8 mL of FRAP reagent, 180 μL of distilled water, and 60 μL of sample were added to the same test tube and incubated at 37 °C for 4 min; absorbance was measured at 593 nm, and the FRAP working solution was used as a blank. Data were calculated according to the following equation that was obtained with FeSO₄ from a calibration curve and then expressed as mmol FeSO₄ per g dry weight (mmol FeSO₄/g DW).

**Extraction of soluble and bound phenolic compounds**

Three solvents were used for comparison of extraction efficiency: (1) ethanol, (2) acetone and (3) water at different temperatures (50, 60, 70, and 80 °C). The phenolic compounds in the test samples were extracted using a modification of the procedure described by Uzelac et al. (2005). Each sample (1 g) was mixed with 10 mL of each solvent in an inert atmosphere (N₂) for 2 h in the dark. The extract was then centrifuged at 2500 rpm/min, and the supernatant was evaporated to dryness under reduced pressure (35–40 °C). For bound phenolic compounds extraction, the bound phenolic contents were extracted according to the method of Butsat et al. (2009) with minor modifications. Briefly, the residue from the 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. Two extracts (soluble and bound fractions) obtained were determined the total phenolic content (TPC), total flavonoid content (TFC) and individual phenolic compounds using HPLC.

**Determination of TPC**

The TPC was determined using the Folin–Ciocalteu reagent as descried by Abu Bakar et al. (2009). Briefly, 300 μL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 mL of sodium carbonate (60 g/L) solution was added to the mixture. After 90 min at room temperature, the absorbance was measured at 725 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

**Determination of TFC**

The TFC was determined using the colorimetric method described by Abu Bakar et al. (2009) with a slight modification. Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by the addition of 0.15 mL of 5 % NaNO₂ solution. After 6 min, 0.3 mL of a 10 % AlCl₃·6H₂O solution was added and allowed to stand for another 5 min before 1.0 mL of 1 M NaOH was added. The mixture was mixed by a vortex mixer. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

**HPLC–DAD system for analysis of phenolic compounds**

HPLC analysis was performed using LC-20AC pumps and SPD-M20A diode array detection (Shimadzu, Japan), while chromatographic separations were performed on a column, Inertsil ODS-3, C18 (4.6 mm x 250 mm, 5 μm) (Hichrom Limited, UK). The compositions of the solvents and gradient elution conditions were described previously by Uzelac et al. (2005) and used with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. The 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. Operation conditions were as follows: column temperature, 38 °C; injection volume, 20 μL; and UV-diode array detection at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonoids) at a flow-rate of 0.8 mL/min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds, and they were detected using the external standard method.
Extraction and determination of \(\gamma\)-oryzanol and tocopherols contents

For extraction, one-step equilibrium direct-solvent extraction was conducted using the method of Butsat and Siriamornpun (2010) with some modifications. Each sample (1 g) was extracted with three solvents: (1) ethanol, (2) acetone, and (3) water at different temperatures (50, 60, 70, and 80 °C), vortexed at maximum speed for 1 min and then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residual was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate.

The contents of \(\gamma\)-oryzanol and tocopherols were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 μm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm × 250 mm, 5 μm) with a mobile phase of acetonitrile/methanol (25:75, v/v), flow rate 1.5 mL/min, and photodiode-array detector at 292 nm for the analysis of tocopherols and at 325 nm for the analysis of \(\gamma\)-oryzanol. Calibration curves were constructed with external standards.

Statistical analysis

Analysis of variance (ANOVA) was performed in a completely randomized design, using Duncan’s Multiple Range Test. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95 % (\(p < 0.05\)).

Results and discussion

Solvents are commonly employed for the extraction of the bioactive compounds from biomaterials. It has been reported that antioxidant compounds and antioxidant activities of the extracts are strongly associated with the solvent used for the extraction, due to the different chemical properties, such as polarity, of compounds obtained from the extract (Moure et al. 2001; Pokorny and Korczak 2001). Therefore, the selection of a solvent that is suitable for the extraction system is extremely important since the structure and matrix composition of each matrix-solvent system can be diverse (Al-Farsi and Lee 2008). For this reason, in this study, various extraction solvents were evaluated for the extraction of rice bran, rice husk, and ground rice husk. The parameters evaluated included antioxidant activities, total phenolic compounds, \(\gamma\)-oryzanol, and tocopherols in the extracts. All data obtained from each extracted sample was statistically compared.

Antioxidant properties, total phenolic and total flavonoid contents

Table 1 shows the influence of solvent extraction on antioxidant activities, as determined using DPPH and FRAP methods of rice bran, rice husk, and ground rice husk. The results showed that ground rice husk had the higher values of DPPH radical scavenging (70–90 %) compared to rice bran (69–87 %) and rice husk (41–87 %), while rice bran had higher FRAP values (10–28 mmol FeSO\(_4\)/g) than ground rice husk (7–19 mmol FeSO\(_4\)/g) and rice husk (5–13 mmol FeSO\(_4\)/g) (Table 1). The solvents used for the extraction of rice bran and rice husk showed significantly different antioxidant activities (\(p < 0.05\)). The antioxidant activities (DPPH and FRAP values) increased in the following order: acetone < ethanol < water. Antioxidant activity depended on the polarity of the solvent, in such a way that when the polarity of the solvent decreased (water > ethanol > acetone), antioxidant activity decreased in the same order. From the principle that polar compounds dissolve polar compounds (Chew et al. 2011), the results could indicate that the phenolic compounds found in rice bran and rice husk have polar characteristics. However, the lowest temperature (50 °C) of the water extracted from the rice bran contained the lowest values of DPPH radical scavenging capacity compared to the other extracts. Extraction temperature is an important factor for the solubility and diffusion coefficient of the solute. In the case of water extraction, antioxidant activity increased when the extraction temperature increased from 50 to 70 °C and then decreased slightly at 80 °C (Table 1). Previous studies have reported a relationship between temperature of extraction and content of bioactive compounds from several sources, such as green tea (Vuong et al. 2011), papaya leaf (Vuong et al. 2013), grape pomace (Pinelo et al. 2005), peanut skins (Ballard et al. 2009), Pyracantha fortuneana fruit (Zhao et al. 2013) and olive seeds (Alu’datt et al. 2011). For example, to obtain the highest levels of phenolic content and antioxidant activity, the extraction temperature should be 70 °C for papaya leaf (Vuong et al. 2013) and olive seeds (Alu’datt et al. 2011), while for extracting catechin from green tea the extraction temperature should be 80 °C (Vuong et al. 2011). During extraction the rates of mass transfer typically increased with higher temperatures of the solvent, and in the case of phenolic compounds, the raised temperatures caused the decomposition and epimerization of the compounds (Gertenbach 2001). We found that the bound extracts exhibited greater radical scavenging activities and reducing power of the antioxidant capacity than those of the soluble extracts in all samples (Table 1). However, the values of the bound forms showed no significant differences among all fractions (\(p < 0.05\)) studied.
Table 1 Effects of extraction solvents on antioxidant activities of rice bran, rice husk, and ground rice husk

| Sample          | DPPH (％ inhibition) | FRAP (μmol FeSO₄/g DW) |
|-----------------|----------------------|------------------------|
|                 | Soluble | Bound | Soluble | Bound |
| Rice bran       |         |       |         |       |
| Ethanol         | 78.66 ± 0.14<sup>c</sup> | 90.47 ± 0.18<sup>b</sup> | 12.94 ± 0.14<sup>c</sup> | 31.62 ± 0.04<sup>a</sup> |
| Acetone         | 69.76 ± 1.16<sup>b</sup> | 90.41 ± 0.14<sup>b</sup> | 10.68 ± 0.16<sup>a</sup> | 31.63 ± 0.06<sup>a</sup> |
| Water 50 °C     | 75.73 ± 0.43<sup>b</sup> | 90.34 ± 0.16<sup>a</sup> | 18.79 ± 0.35<sup>c</sup> | 31.61 ± 0.03<sup>a</sup> |
| Water 60 °C     | 82.97 ± 1.77<sup>d</sup>,<sup>b</sup> | 90.53 ± 0.12<sup>b</sup> | 27.17 ± 0.14<sup>a</sup> | 31.60 ± 0.04<sup>a</sup> |
| Water 70 °C     | 87.93 ± 0.05<sup>b</sup> | 90.38 ± 0.24<sup>b</sup> | 28.57 ± 0.36<sup>b</sup> | 31.65 ± 0.07<sup>a</sup> |
| Water 80 °C     | 78.75 ± 0.78<sup>c</sup> | 90.42 ± 0.28<sup>a</sup> | 28.24 ± 0.15<sup>a</sup> | 31.57 ± 0.11<sup>a</sup> |
| Rice husk       |         |       |         |       |
| Ethanol         | 41.41 ± 0.19<sup>b</sup> | 87.62 ± 0.25<sup>a</sup> | 5.66 ± 0.39<sup>c</sup> | 14.84 ± 0.07<sup>c</sup> |
| Acetone         | 77.01 ± 0.29<sup>b</sup> | 87.56 ± 0.37<sup>a</sup> | 5.23 ± 0.09<sup>c</sup> | 14.86 ± 0.04<sup>c</sup> |
| Water 50 °C     | 68.63 ± 0.37<sup>b</sup> | 87.50 ± 0.33<sup>c</sup> | 5.71 ± 0.12<sup>c</sup> | 14.85 ± 0.06<sup>c</sup> |
| Water 60 °C     | 78.69 ± 0.23<sup>c</sup> | 87.59 ± 0.28<sup>a</sup> | 11.37 ± 0.18<sup>b</sup> | 14.88 ± 0.09<sup>c</sup> |
| Water 70 °C     | 87.10 ± 0.38<sup>b</sup> | 87.57 ± 0.23<sup>a</sup> | 13.52 ± 0.13<sup>c</sup> | 14.82 ± 0.11<sup>c</sup> |
| Water 80 °C     | 81.17 ± 0.28<sup>b</sup> | 87.65 ± 0.24<sup>a</sup> | 12.96 ± 0.17<sup>b</sup> | 14.86 ± 0.10<sup>c</sup> |
| Ground rice husk|         |       |         |       |
| Ethanol         | 83.74 ± 0.23<sup>c</sup> | 93.22 ± 0.18<sup>a</sup> | 8.47 ± 0.08<sup>b</sup> | 23.78 ± 0.05<sup>b</sup> |
| Acetone         | 70.19 ± 0.60<sup>b</sup> | 93.13 ± 0.28<sup>a</sup> | 7.66 ± 0.18<sup>c</sup> | 23.82 ± 0.07<sup>b</sup> |
| Water 50 °C     | 76.49 ± 0.38<sup>b</sup>,<sup>c</sup> | 93.16 ± 0.19<sup>a</sup> | 12.15 ± 0.11<sup>b</sup> | 23.76 ± 0.13<sup>b</sup> |
| Water 60 °C     | 90.47 ± 0.49<sup>b</sup> | 93.04 ± 0.18<sup>a</sup> | 18.94 ± 0.41<sup>c</sup> | 23.80 ± 0.06<sup>b</sup> |
| Water 70 °C     | 90.86 ± 0.82<sup>a</sup> | 93.37 ± 0.35<sup>a</sup> | 19.66 ± 0.16<sup>c</sup> | 23.79 ± 0.04<sup>b</sup> |
| Water 80 °C     | 82.15 ± 0.51<sup>c</sup>,<sup>e</sup>,<sup>b</sup> | 93.13 ± 0.20<sup>a</sup> | 19.75 ± 0.37<sup>b</sup> | 23.77 ± 0.11<sup>b</sup> |

DPPH 2,2-difenyl-1-picrylhidrazyl radical scavenging activity, FRAP ferric reducing antioxidant power

Values are expressed as mean ± standard deviation (n = 3).

A, B, C Values in the same column followed by different letters are significantly different (p < 0.05).

a, b, c Values in the same row followed by different letters are significantly different (p < 0.05).

Total soluble and bound phenolic as well as total flavonoid contents of rice bran, rice husk, and ground rice husk as affected by different solvents are presented in Table 2. Rice bran contained higher values of TPC (1.20–4.29 mg GAE/g dry sample) than ground rice husk (1.17–2.24 mg GAE/g dry sample) and rice husk (0.63–1.46 mg GAE/g dry sample). The solvents used for the extraction significantly affected the TPC and TFC of the rice bran and rice husk (p < 0.05), depending on the polarity of the solvent, in that when the polarity of the solvent decreased (water > ethanol > acetone), they increased in the following order: acetone < ethanol < water (at different temperatures). Many studies have reported that the contents of phenolic in various plants and antioxidant activities are related with the polarity of extraction solvents (Park et al. 2011; Yang and Lee 2012; Luyen et al. 2014; Hyun et al. 2015). In the case of water extraction, TPC increased as the temperature increased. The high temperature used in the extraction with water could also explain the high extraction efficiency obtained. The extraction temperature is an important factor related to the solute’s solubility and diffusion coefficient. A high temperature could also promote the destruction of the matrix tissues so more compounds could be released into the solvent (Al-Farsi and Lee 2008). The highest concentration of bound TPC was observed in ground rice husk, while bound TPC was lowest in rice husk. On average, bound TPC was three times higher than soluble TPC in all fractions.

The acetone extraction of all samples contained the lowest amount of TFC compared to other extracted samples. In the case of water extraction, TFC increased when the extraction temperature increased from 50 to 70 °C and then decreased slightly at 80 °C. The TFC varied significantly (p < 0.05) between the soluble and bound fractions for all samples in this study (Table 2). In general, all soluble extracts had higher TFCs than their corresponding bound extracts. Previous studies on the extraction of soluble and bound TFC from edible flowers (Kaisoon et al. 2011) and millet (Chandrakarsha and Shahidi 2010) have reported that soluble extracts had higher TFCs than bound extracts. In contrast, Adom and Liu (2002) reported that soluble extracts of corn, wheat, oat, and rice contained less TFC than their bound counter parts. The increase of TPC and TFC in ground rice husk may be caused by the particle
size being reduced. There were a few previous studies on the effects of particle size reduction for bioactive compound extraction from various sources, such as wheat bran, black currant pomace, and black cohosh (Landbo and Meyer 2001; Mukhopadhyay et al. 2006; Brewer et al. 2014).

### Individual phenolic acids and flavonoids

Phenolic acids can be classified as free, soluble conjugated, and bound (Regnier and Macheix 1996). Our present study determined that there were both free and bound phenolic acids in the rice bran, rice husk, and ground rice husk. It was possible to identify four hydroxybenzoic acids (HBA): gallic acid, protocatechuic acid, p-hydroxybenzoic acid and vanillic acid; and six hydrocinamic acids (HCA): chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid. It was possible to identify five flavonoids (rutin, myricetin, quercetin, apigenin, and kaempferol). Changes in compositions and concentrations of these components were affected by different solvent extractions. For soluble phenolic acids analysis, the identification and quantification of the major phenolic compounds present in the various extracts are shown in Table 3. The main soluble phenolic acids found in all samples were ferulic, protocatechuic, gallic, and vanillic acids. On comparing the phenolic acids of all samples, the rice bran contained the highest levels of protocatechuic acid, with concentrations ranging from 1.8 to 12.7 mg/g.

### Table 2 Effects of extraction solvents on total phenolic and total flavonoid contents of rice bran, rice husk, and ground rice husk

| Sample          | TPC (mg GAE/g DW) | TFC (mg RE/g DW) |
|-----------------|-------------------|------------------|
|                 | Soluble           | Bound            | Soluble           | Bound            |
| Rice bran       |                   |                  |                   |                  |
| Ethanol         | 2.39 ± 0.04³, B   | 7.78 ± 0.11³, A  | 3.25 ± 0.05³, A   | 2.57 ± 0.03³, B  |
| Acetone         | 1.20 ± 0.03³, L, B| 7.76 ± 0.16³, A  | 1.52 ± 0.04³, B   | 2.55 ± 0.04³, A  |
| Water 50 °C     | 3.26 ± 0.07³, L   | 8.08 ± 0.34³, b, A| 3.36 ± 0.04³, A   | 2.55 ± 0.06³, A  |
| Water 60 °C     | 3.36 ± 0.01³, B   | 8.00 ± 0.07³, b, c, A| 3.72 ± 0.04³, A   | 2.58 ± 0.00³, B  |
| Water 70 °C     | 3.52 ± 0.06³, B   | 7.94 ± 0.03³, b, c, A| 3.88 ± 0.09³, A   | 2.57 ± 0.04³, B  |
| Water 80 °C     | 4.29 ± 0.01³, B   | 7.79 ± 0.12³, c, A| 3.87 ± 0.08³, A   | 2.56 ± 0.03³, B  |
| Rice husk       |                   |                  |                   |                  |
| Ethanol         | 0.92 ± 0.03³, B   | 2.28 ± 0.09³, A  | 2.04 ± 0.09³, A   | 1.29 ± 0.05³, B  |
| Acetone         | 0.79 ± 0.01³, B   | 2.20 ± 0.03³, A  | 0.32 ± 0.08³, B   | 1.28 ± 0.04³, A  |
| Water 50 °C     | 0.63 ± 0.02³, B   | 2.27 ± 0.03³, A  | 1.17 ± 0.09³, A   | 1.30 ± 0.03³, B  |
| Water 60 °C     | 0.87 ± 0.02³, B   | 2.29 ± 0.04³, A  | 2.54 ± 0.10³, A   | 1.29 ± 0.03³, B  |
| Water 70 °C     | 1.12 ± 0.01³, B   | 2.28 ± 0.04³, A  | 2.66 ± 0.11³, A   | 1.31 ± 0.02³, B  |
| Water 80 °C     | 1.46 ± 0.02³, B   | 2.25 ± 0.01³, A  | 2.65 ± 0.09³, A   | 1.29 ± 0.03³, B  |
| Ground rice husk|                   |                  |                   |                  |
| Ethanol         | 2.24 ± 0.03³, B   | 8.19 ± 0.24³, A  | 3.16 ± 0.0 and 16³, A| 1.50 ± 0.03³, B  |
| Acetone         | 1.18 ± 0.03³, B   | 7.94 ± 0.17³, b, c, A| 1.44 ± 0.13³, A   | 1.28 ± 0.03³, B  |
| Water 50 °C     | 1.23 ± 0.03³, B   | 8.14 ± 0.26³, A  | 3.27 ± 0.22³, A   | 1.29 ± 0.02³, B  |
| Water 60 °C     | 1.27 ± 0.02³, B   | 8.13 ± 0.16³, A  | 3.63 ± 0.24³, A   | 1.30 ± 0.02³, B  |
| Water 70 °C     | 1.68 ± 0.00³, B   | 8.15 ± 0.21³, A  | 3.76 ± 0.29³, A   | 1.29 ± 0.04³, B  |
| Water 80 °C     | 2.02 ± 0.01³, B   | 8.16 ± 0.20³, A  | 3.74 ± 0.23³, A   | 1.33 ± 0.04³, B  |

Values are expressed as mean ± standard deviation (n = 3)

GAE, gallic acid equivalents, RE rutin equivalents, DW dry weight

a, b, c Values in the same column followed by different letters are significantly different (p < 0.05)

A, B, C Values in the same row followed by different letters are significantly different (p < 0.05)
### Table 3: Effects of extraction solvents on soluble phenolic acids (µg/g dry weight) of rice bran, rice husk, and ground rice husk

| Sample          | Soluble hydrobenzoic acids (µg/g DW) | Soluble hydrocinnamic acids (µg/g DW) | Total |
|-----------------|--------------------------------------|---------------------------------------|-------|
|                 | GA                                   | PCCA                                  | ChA   |
| Rice bran       |                                      |                                       |       |
| Ethanol         | 1.69 ± 0.01<sup>i</sup>              | 1.79 ± 0.02<sup>e</sup>              | ND    |
| Acetone         | ND                                   | ND                                    | ND    |
| Water 50 °C     | 3.90 ± 0.02<sup>ef</sup>             | 7.34 ± 0.07<sup>d</sup>              | ND    |
| Water 60 °C     | 4.08 ± 0.03<sup>c</sup>              | 3.62 ± 0.06<sup>d</sup>              | ND    |
| Water 70 °C     | 5.47 ± 0.41<sup>c</sup>              | 12.69 ± 0.48<sup>a</sup>             | ND    |
| Water 80 °C     | 4.60 ± 0.04<sup>d</sup>              | 12.23 ± 0.48<sup>b</sup>             | ND    |
| Rice husk       |                                      |                                       |       |
| Ethanol         | 0.85 ± 0.01<sup>j</sup>              | 1.73 ± 0.00<sup>f</sup>              | ND    |
| Acetone         | ND                                   | ND                                    | ND    |
| Water 50 °C     | 2.95 ± 0.05<sup>h</sup>              | 3.57 ± 0.02<sup>ef</sup>             | ND    |
| Water 60 °C     | 3.03 ± 0.04<sup>bf</sup>             | 3.66 ± 0.03<sup>c</sup>              | ND    |
| Water 70 °C     | 3.78 ± 0.07<sup>if</sup>             | 3.40 ± 0.01<sup>ef</sup>             | ND    |
| Water 80 °C     | 3.22 ± 0.15<sup>g</sup>              | 3.29 ± 0.01<sup>f</sup>              | ND    |
| Ground rice husk|                                      |                                       |       |
| Ethanol         | 1.74 ± 0.01<sup>i</sup>              | 1.84 ± 0.02<sup>e</sup>              | ND    |
| Acetone         | ND                                   | ND                                    | ND    |
| Water 50 °C     | 3.83 ± 0.06<sup>ef</sup>             | 3.60 ± 0.03<sup>ef</sup>             | ND    |
| Water 60 °C     | 4.42 ± 0.04<sup>bd</sup>             | 9.41 ± 0.32<sup>c</sup>              | ND    |
| Water 70 °C     | 8.72 ± 0.07<sup>a</sup>              | 3.48 ± 0.02<sup>ef</sup>             | ND    |
| Water 80 °C     | 6.47 ± 0.23<sup>b</sup>              | 3.69 ± 0.01<sup>c</sup>              | ND    |

Values are expressed as mean ± standard deviation (n = 3)

Means with different letters in the same column were significantly different at the level p < 0.05

ND not detected, GA gallic acid, PCCA protocatechuic acid, p-OH, p-hydroxybenzoic acid, VA vanillic acid, ChA chlorogenic acid, CFA caffeic acid, SyA syringic acid, p-CA p-coumaric acid, FA ferulic acid, SNA sinapic acid
higher contents of HBA (12 and 22 μg/g DW), respectively (data not shown). The 70 °C water-extracted rice husk, and ground rice husk had higher contents of HBA (12 and 22 μg/g DW) than did the 70 °C water (11 and 20 μg/g DW), 50 °C water (13 and 17 μg/g DW), 80 °C water (10 and 16 μg/g DW) and ethanol (7 and 9 μg/g DW), respectively (data not shown).

In addition, we found that HBA was not detected in the acetone extracted samples. On the other hand, bound phenolic acids are typically part of the cell wall structure (Bunzel et al. 2002) where the cross-linking esters of lignin compositions and concentrations of phenolic acids and flavonoids in rice bran and rice husk were influenced by the different solvent extraction systems. For bound flavonoids analysis, the contents of bound flavonoids are listed in Table 4. With respect to variation in the contents of bound flavonoids in all extracts, ground rice husk exhibited the highest total bound flavonoids (10 μg/g DW). Compared to soluble flavonoids, the contents of bound flavonoids were lower, except for the presence of apigenin in the bound extracts. Like most flavones, apigenin is usually found in plants bound to sugars as glycosides or in an ester form with tannin acid. Apigenin is a 4’,5,7 trihydroxy flavone, which belongs to a less toxic and non-mutagenic flavone subclass of flavonoids. It has a variety of pharmacological activities, including antioxidant (Skerget et al. 2005), antibacterial (Martini et al. 2004), anti-inflammatory (Hougeea et al. 2005), antiproliferative (Kim et al. 2005), anti-tumor (Wang et al. 2005), oxygenase inhibitor (Abate et al. 2005) and induction of apoptosis (Tajdar and Sarwat 2006). Apigenin has gained particular interest in recent years as a beneficial and health-promoting agent due to its

### Table 4 Bound phenolic acids (μg/g dry weight) of rice bran, rice husk, and ground rice husk

| Bound phenolics (μg/g DW) | Sample                  |
|--------------------------|-------------------------|
|                          | Rice bran | Rice husk | Ground rice husk |
| Hydrobenzoic acids       |           |           |                 |
| Gallic acid              | 7.11 ± 0.03<sup>b</sup> | 5.52 ± 0.03<sup>c</sup> | 13.94 ± 0.10<sup>a</sup> |
| Protocatechuic acid      | 13.96 ± 0.03<sup>a</sup> | 4.25 ± 0.02<sup>c</sup> | 9.66 ± 0.08<sup>b</sup> |
| p-hydroxybenzoic acid    | 2.89 ± 0.03<sup>c</sup> | 5.15 ± 0.01<sup>b</sup> | 5.88 ± 0.02<sup>a</sup> |
| Vanillic acid            | 14.13 ± 0.18<sup>a</sup> | 5.04 ± 0.05<sup>c</sup> | 7.99 ± 0.13<sup>b</sup> |
| Hydrocinnamic acids      |           |           |                 |
| Chlorehogenic acid       | 5.65 ± 0.07<sup>a</sup> | 3.06 ± 0.05<sup>c</sup> | 3.34 ± 0.04<sup>b</sup> |
| Caffeic acid             | 2.37 ± 0.02<sup>c</sup> | 4.53 ± 0.02<sup>b</sup> | 8.69 ± 0.04<sup>a</sup> |
| Syringic acid            | 18.61 ± 0.07<sup>c</sup> | 22.07 ± 0.09<sup>b</sup> | 26.83 ± 0.06<sup>a</sup> |
| p-coumaric acid          | 132.61 ± 0.73<sup>c</sup> | 201.15 ± 1.06<sup>b</sup> | 287.64 ± 0.74<sup>a</sup> |
| Ferulic acid             | 44.89 ± 0.17<sup>c</sup> | 71.88 ± 0.27<sup>b</sup> | 97.63 ± 0.86<sup>a</sup> |
| Sinapic acid             | 3.39 ± 0.05<sup>c</sup> | 5.35 ± 0.06<sup>b</sup> | 7.24 ± 0.07<sup>a</sup> |
| Total phenolic acids     | 245.62 ± 0.64<sup>c</sup> | 328.09 ± 1.47<sup>b</sup> | 468.86 ± 1.37<sup>a</sup> |

Values are expressed as mean ± standard deviation (n = 3)

Means with different letters in the same row were significantly different at the level p < 0.05
Table 5  Effects of extraction solvents on soluble flavonoids (µg/g dry weight) of rice bran, rice husk, and ground rice husk

| Sample          | Soluble flavonoid contents (µg/g DW) | Total  |
|-----------------|--------------------------------------|--------|
|                 | Rutin | Myricetin | Quercetin | Apigenin | Kaempferol |
| Rice bran       |       |           |           |          |            |
| Ethanol         | ND    | ND        | ND        | ND       | 1.99 ± 0.04b | 1.99 ± 0.04m |
| Acetone         | ND    | ND        | ND        | ND       | 1.06 ± 0.00c | 1.06 ± 0.00p |
| Water 50 °C     | ND    | ND        | ND        | ND       | 1.38 ± 0.01d | 1.38 ± 0.01e |
| Water 60 °C     | ND    | ND        | ND        | ND       | 1.71 ± 0.02c | 1.71 ± 0.02a |
| Water 70 °C     | ND    | ND        | ND        | ND       | 2.08 ± 0.02a | 2.08 ± 0.02 l |
| Water 80 °C     | ND    | ND        | ND        | ND       | 2.08 ± 0.02a | 2.08 ± 0.02 l |
| Rice husk       |       |           |           |          |            |
| Ethanol         | ND    | 25.97 ± 0.10e^c | ND        | ND       | 25.97 ± 0.10f |
| Acetone         | ND    | 13.00 ± 0.02i | ND        | ND       | 19.40 ± 0.03g |
| Water 50 °C     | ND    | 19.40 ± 0.03g | ND        | ND       | 19.40 ± 0.03g |
| Water 60 °C     | ND    | 25.87 ± 0.10e | ND        | ND       | 25.87 ± 0.10e |
| Water 70 °C     | ND    | 27.75 ± 0.11d | ND        | ND       | 27.75 ± 0.11d |
| Water 80 °C     | ND    | 27.75 ± 0.11d | ND        | ND       | 27.75 ± 0.11d |
| Ground rice husk|       |           |           |          |            |
| Ethanol         | ND    | 32.43 ± 0.10c | ND        | ND       | 32.43 ± 0.10c |
| Acetone         | ND    | 11.20 ± 0.06j | ND        | ND       | 16.16 ± 0.11b |
| Water 50 °C     | ND    | 16.16 ± 0.11b | ND        | ND       | 16.16 ± 0.11b |
| Water 60 °C     | ND    | 25.09 ± 0.21f | ND        | ND       | 25.09 ± 0.21f |
| Water 70 °C     | ND    | 32.62 ± 0.10b | ND        | ND       | 32.62 ± 0.10b |
| Water 80 °C     | ND    | 33.22 ± 0.15a | ND        | ND       | 33.22 ± 0.15a |

Values are expressed as mean ± standard deviation (n = 3)
Means with different letters in the same column were significantly different at the level p < 0.05
ND not detected

Table 6  Bound flavonoids (µg/g dry weight) of rice bran, rice husk, and ground rice husk

| Bound flavonoids (µg/g DW) | Sample          | Rice bran | Rice husk | Ground rice husk |
|---------------------------|-----------------|-----------|-----------|------------------|
|                           | Rutin | Myricetin | Quercetin | Apigenin | Kaempferol |
| Rice bran                 | ND    | ND        | ND        | ND       | ND          |
| Rice husk                 | ND    | 6.66 ± 0.08b | 8.80 ± 0.08a | ND      | ND          |
| Ground rice husk          | ND    | 0.53 ± 0.03b | 0.82 ± 0.02a | ND      | ND          |
|                           | 0.52 ± 0.04 | ND        | ND        | ND      | ND          |
|                           | 0.66 ± 0.02c | 7.27 ± 0.03b | 9.63 ± 0.11a | ND      | ND          |

Values are expressed as mean ± standard deviation (n = 3)
Means with different letters in the same row were significantly different at the level p < 0.05
ND not detected

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low intrinsic toxicity and striking effects on normal versus cancer cells, compared with other structurally related flavonoids (Shukla and Gupta 2010). Apigenin is a strong inhibitor of ornithine decarboxylase, which is an enzyme that plays a major role in tumor promotion (Wei et al. 1990). In addition, apigenin has been shown to increase the intracellular concentration of glutathione, which enhances the endogenous defense against oxidative stress (Myhrstad et al. 2002).

The uses of different solvents in the extraction process were anticipated to lead to different total phenolic values, which would be due to the different efficacies of the phenolic compounds’ solubilization and the sample matrixes’ penetration. In general, it was observed that increased temperatures at a moderate level, between 50 and 70 °C, provided a positive effect on the efficiency and rate of the extraction in our study. The degradation or epimerization of some of the total phenolic compounds in the temperature
range investigated was observed at a temperature above 70 °C, as some of the functional components may deteriorate with temperature. An increase in solvent temperatures normally increases the mass transfer rates during extraction, and in the case of phenolic compounds, elevated temperatures activate competing processes, such as decomposition and epimerization of the compounds (Gertenbach 2001).

**γ-Oryzanol and tocopherols**

The γ-oryzanol and tocopherols are bioactive compounds present in rice bran oil. They are actively involved in lipophilic antioxidants due to their solubility in the lipid phase. In fact, γ-oryzanol and tocopherols are insoluble in water because they have long polar chains. However, the experiment was performed to compare the results of the extraction by the solvents utilized. The effects of different extraction solvents on γ-oryzanol and tocopherols content in rice bran, rice husk, and ground rice husk extracts are shown in Fig. 1. The results showed that γ-oryzanol and tocopherols content were the highest with acetone extracts, followed by ethanol, while no γ-oryzanol and tocopherols were detected in the water extracts.

Figure 1A shows the effects of different extraction solvents on γ-oryzanol in rice bran, rice husk, and ground rice husk extracts. For all sample extracts, the amount of γ-oryzanol ranged from 0.028 to 5.701 mg/g. Rice bran was the best source of γ-oryzanol (3.013–5.701 mg/g), followed by ground rice husk (0.120–0.979 mg/g) and finally rice husk (0.028–0.570 mg/g). Variations in the γ-oryzanol content of the three extracts are attributed to the polarities of different solvents used in the experiment. The results showed that the acetone extracted rice bran had the highest γ-oryzanol content (5.701 mg/g), followed by ethanol (3.013 mg/g) (Fig. 1A), which agreed with the results from barley (Liu and Yao 2007) and buckwheat (Sun and Ho 2005). The results indicated that acetone may be a better choice for extracting γ-oryzanol from rice bran and rice husk.

The effects of different extraction solvents on tocopherols contents from rice bran, rice husk, and ground rice husk are shown in Fig. 1B. γ-Tocopherol was only found in rice bran, and it was not detected in rice bran and rice husk. The acetone-extracted rice bran had higher α- and γ-tocopherols contents (82.15 and 5.04 μg/g DW) than the ethanolic extracts (28.12 and 1.27 μg/g DW). Based on the obtained results, it can be inferred that acetone is the best solvent for tocopherols extraction in terms of quantity due to its polarity properties. Similar to the results of TPC and TFC, ground rice husk contained greater levels of γ-oryzanol than native rice husk. Presumably, when the particle size is reduced, the accessible surface for the extractive solvent to attack is increased, which results in the observed γ-oryzanol content of the ground rice husk. One of the major limiting steps in the solid/liquid extraction is the diffusion to the surface of the solid (Vandenburg et al. 1997). The smaller particle sizes generally have lower intra-particle diffusion resistance because of their shorter diffusion paths. Besides, smaller particle sizes would provide a greater surface area and their cell walls could have been broken to a greater degree during grinding (Jeng et al. 2013). Therefore, the greater recovery of γ-oryzanol from ground rice husk was anticipated.

In summary, our study has demonstrated that various solvents influenced the contents and compositions of phenolic compounds and the antioxidant activities of the extracts of rice bran and rice husk. Overall, the rice by-products extracted with heated water extracts had higher contents of bioactive compounds, such as phenolic acids, flavonoids, and antioxidant activities, compared with those extracted with acetone and ethanol. The total phenolic acids contents of bound extracts were greater than those of soluble extracts in all samples studied. However, the organic solvents, especially acetone, were considered to be the most effective for extracting γ-oryzanol and tocopherols from rice bran and husk. In addition, decreasing the
particle size in the husk was found to work positively to enhance antioxidant activities, γ-oryzanol, and phenolic compounds. Our results reveal that the extraction of bioactive compounds from rice by-products, to be used as antioxidants, produced various quantities and compositions that depended on the characteristic properties of the extraction solvents. The results obtained demonstrated the potentials of the rice bran and rice husk as natural sources of antioxidant properties and phenolic compounds that bring health benefits from the powerful active ingredients with applications in the manufacture of nutritional supplements, health industries, herbal pharmaceutical compounds, and other possible applications.

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