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Effects of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk

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ABSTRACT

We investigated the changes of antioxidant activity and bioactive compounds in bran, rice husk and ground rice husk after three different treatments, namely hot-air, far-infrared radiation (FIR), and cellulase, compared with raw samples. Overall, FIR-treated group showed a higher DPPH radical scavenging activities, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC) than did hot-air and cellulase treatments for all samples. A significant increase in α- and γ-tocopherols was found in FIR irradiated rice bran compared to raw bran, while α- and γ-tocopherols in hot-air and cellulase treated rice bran were remained unchanged. Cellulase significantly increased the amount of vanillic acid; however a dramatic decrease of ferulic acid was observed. The contents of γ-oryzanol in cellulase treated ground rice husk were significantly increased. Decreasing particle size in the husk was found to work positively for enhancing antioxidant activities, γ-oryzanol and phenolic compounds.

1. Introduction

Rice is a staple food in many parts of the world. Rice is the main agricultural product produced in Thailand and exported to other countries. Approximately 21–26 million tons of rice are annually produced (OAE, 2003). Cereal grains, especially rice, contain special phenolic acids (such as ferulic acid, *p*-coumaric and diferulate) that are not present in significant quantities in fruit and vegetables (Adom & Liu, 2002). Most of these compounds are found in different parts of cereal grains, particularly in distinct fractions from milling the grains (Onyeneho & Hettiarachchy, 1992). Rice milling waste is the by-product obtained from small-scale rice mills. The rice milling waste fractions obtained contains husks, bran, polishing and small quantity of broken rice (Onyeneho & Hettiarachchy, 1992) and are cheap as they are discarded as waste. The commercial rice-milling process leads to products with low-value fractions, such as husk and bran. Rice bran is a rich source of oryzanols or steryl ferulate esters (Norton, 1995). In addition, rice bran is a potential source of tocopherols, tocotrienols and phenolic compounds which have shown antioxidant activity (Nicolosi, Rogers, Ausman, & Orthoefer 1994). Because rice husks are inedible, they are used in various non-food applications, such as low-value waste materials. However, rice husks offer the valuable nutritional advantage that they contain an antioxidant-defence system to protect the rice seed from oxidative stress (Ramarathnam, Osawa, Namiki, & Kawakishi 1988). However, most antioxidative phenolic compounds in plants are found in a form of – covalently bound with insoluble polymer (Niwa & Miyachi, 1986). Therefore it is necessary to find an effective processing method to release those compounds. Several methods such as heat treatment, far-infrared (FIR) radiation, and enzymatic treatment have been studied to liberate and activate low molecular weight natural antioxidants for various agricultural products such as barley (Duh, Yen, Yen, & Chang, 2001) and rice hulls (Lee et al., 2003).Therefore, the aim of the present study was to assess the influence of different treatments on the antioxidant properties and phenolic compounds from rice by-products, namely rice bran and husk.

2. Materials and methods

2.1. Sample preparation

Paddy-rice samples (KDML 105 variety) were obtained from 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 was called ground rice husk. Moisture

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was determined by drying at 110 °C to constant mass. This and all other analyses were performed using triplicate samples and analytical results were expressed on a dry matter basis. The samples were stored at −20 °C prior to analysis.

2.2. Pretreatment process

2.2.1. Hot-air and FIR treatment

Samples were subject to two different treatments, i.e., hot-air and FIR. For each treatment, 100 g of raw samples were used. The protocols used in this present study were selected from the optimal conditions of each method, which were preliminarily studied in our lab. In hot-air treatment, the sample was treated by hot-air drying machine at 120 °C for 30 min using hot-air oven (UFE 600, Memmert, Memmert Company, Germany). In FIR treatment, the sample was FIR-irradiated in the FIR dryer at FIR intensity of 2 kW/m^2 (FIR energy irradiated per FIR heater surface area). Drying temperature was set at 40 °C and drying time of 2 h.

2.2.2. Enzyme aided

Two grams of samples were incubated with the cellulase mixture. Enzyme hydrolysis experiments were conducted at the conditions of pH and temperature advised by the enzyme manufacture (pH = 5 and T = 50 °C) and reactions were run for 24 h. After the enzymatic treatment, the proper solvent was added and extraction was carried out at the extraction conditions described.

2.3. Chemicals and reagents

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, standards of phenolic compounds (gallic acid (GA), protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-OH), vanilic acid (VA), chorogenic acid (ChA), caffeic acid (CFA), syringic acid (SyA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), sinapic acid (SNA), rutin, myricetin, quercetin, apigenin and kaempferol were obtained from Fluka (Neu-Ulm, Germany). Oryzanol (Food Grade, 99.9% purity) was obtained from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). The acetic acid, methanol, acetonitrile and other solvents and reagents used in the HPLC analysis were purchased from Merck (Darmstadt, Germany). All chemicals and reagents used in the study were of analytical grade.

2.4. Assessment of antioxidant activity

2.4.1. Sample extraction

The extracts prepared from rice bran and rice husk were made by 70 °C of distilled water for 2 h in a thermostated water-bath (UMAX, UM-SW 50L). The ratio between sample and extraction medium was 1:10 (w/v). The mixtures were then filtered through filter paper (Whatman No. 1) and the filtrate used for analyzing antioxidant activity *in vitro*. All analyses were performed in triplicate.

2.4.2. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured as described by Butsat and Siriamornpun (2010) with some modifications. Briefly, sample extract (0.1 mL) was mixed with 1.9 mL of a 0.1 mM DPPH in ethanol. The mixture was vortexed (1 min), left to stand at room temperature in dark (30 min) and then the absorbance of this solution was read at 517 nm. The percent inhibition activity was calculated as $[(Ao - Ae)/Ao] \times 100$ (Ao = absorbance without extract; Ae = absorbance with extract).

2.4.3. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}–Fe^{2+}. The formation of blue coloured Fe^{2+}–TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Kubola and Siriamornpun (2008) was used with some modifications. 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), 10 mL $FeCl_3{\cdot}6H2O$ (20 nM) in a ratio of 10:1:1 and 12 mL distilled water, at 37 °C. To perform the assay, 1.8 mL of FRAP reagent, 180 μL Milli-Q water and 60 μL sample, standard or blank were then added to the same test tubes, and incubated at 37 °C for 4 min; absorbance was measured at 593 nm, using the FRAP working solution as a blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using the $FeSO_4{\cdot}7H2O$ linear regression equation to calculate the FRAP values of the sample.

2.5. Determination of total flavonoid content

Total flavonoid content (TFC) was determined using colorimetric method described by Abu Bakar, Mohamed, Rahmat, and Fry (2009) with slight modification. Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3{\cdot}6H_2O$ 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 vortex mixer. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.6. Extraction and determination of γ-oryzanol and tocopherols contents

One-step equilibrium direct-solvent extraction was conducted by the method of Butsat and Siriamornpun (2010) with some modifications. Each sample (1 g) was extracted with acetone at a ratio of 1:10 w/v, vortexed at maximum speed for 1 min 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 γ-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 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 γ-oryzanol. Calibration curves were constructed with the external standards.

2.7. Identification and quantification of phenolic compounds

2.7.1. Determination of total phenolic content

The total phenolics content (TPC) was determined using the Folin–Ciocalteu reagent as followed 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, 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).