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Phenolic profiles and antioxidant activity in four tissue fractions of whole brown rice

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ABSTRACT

In view of different processing rice types contained different tissue fractions, the present study quantified free and bound phenolic profiles and antioxidant activity in the pericarp, aleurone layer, embryo and endosperm fractions of *japonica* and *indica* whole brown rice. Significant differences were found in the total phenolic contents, oxygen radical absorbance capacity (ORAC) and cellular antioxidant activity (CAA) of the different fractions. The ratios of free and bound phenolics to total were various. Thirteen individual phenolics (gallic, protocatechuic, hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, isoferulic, coumaric and ferulic acids, catechin, epicatechin and quercetin) were detected in both free and bound forms. The contribution of the pericarp, aleurone layer and embryo fractions to the whole brown rice were, respectively, 13.0%, 28.5% and 8.8% for total phenolics, 14.1%, 29.7% and 9.1% for total flavonoids, 18.2%, 38.0%, 11.1% for total ORAC values and 14.6%, 38.0%, 16.9% for CAA values. These findings indicate that the phenolics in brown rice can be concentrated by processing different fractions or to a different milling level because of the uneven distribution of chemical constituents.

Keywords: whole brown rice; tissue fraction; phenolics; flavonoids; phenolic acids; antioxidant activity

1. Introduction

Rice is one of the most important grain crops worldwide. Rice production is the highest of all foodstuffs (total output about 600 million tons) and provides the staple food for more than half of the global population[1]. Rice has two major subspecies; *Oryza sativa* L. *japonica*, mainly consumed in Southeast Asia, Northern China, Japan and the United States, and *Oryza sativa* L. *indica*, mainly consumed in India, Southern China, and Southeast Asia. Whole brown rice is a rice grain from which only the husk has been removed. The hull, the outer covering, corresponds to 18–20% of the total weight of rice. It is removed from the brown rice by dehulling[1]. Rice, like other cereals, does not have a homogeneous structure in the hulled kernel. Instead, the rice kernel is differentiated from its outer surface to its inner central part into four tissue fractions: the pericarp (2–3% of whole brown rice by total weight), the aleurone layer (4–6%), the embryo (2–3%) and the endosperm (about 90%). The endosperm fraction, also called white or polished rice, is a major part of the human daily diet in many countries [2].

Because of the poor sensory quality of brown rice, the embryo and bran layer, including the pericarp and aleurone layer, is removed for human consumption. White rice, which commands a higher price on the market, is obtained after removing 10–15% by total weight from brown rice. However, white rice lacks nutrients as they have been lost from brown rice during the process of milling [3]. Epidemiological studies have associated increased whole grain consumptions with a reduced risk of many chronic diseases. Phenolics are important antioxidants, shown to be responsible

for many health benefits, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [4-8]. The concentrations of total phenolics in rice bran from five rice varieties grown in Southern China were 13.1 times higher than in the endosperm fraction [9]. The higher concentration and activity of phenolics in the rice bran fraction may account for the potentially beneficial medical effects of brown rice. Furthermore, the analysis of successive milling fractions has shown that nutrients are not uniformly distributed in brown rice [10-14]. Rice with different degrees of milling contains different tissue fractions. At present, many types of rice are available on the food markets of Asian countries: semi-brown rice (brown rice with the pericarp/testa removed), embryo rice (brown rice with the pericarp/testa and a small percentage of the aleurone layer removed), lightly milled rice (brown rice with the pericarp/testa, embryo and most of the aleurone layer removed) and polished rice (brown rice with the pericarp/testa, embryo and most of the aleurone layer removed) as well as brown rice.

The contents of brown rice constituents have been analyzed in fractions obtained at different stages of multistep milling. Researchers have previously reported the effect of milling on the nutritional constituents of brown rice, but have focused on minerals, starch and proteins [2, 11-17]. Protein and mineral contents decreased, while starch content increased from the outer bran layers to the endosperm. Data on phytochemical profiles have been limited to a few studies [18, 19]. Phytic acid, Vitamin E and γ-Oryzanol compounds have also been analyzed: Monks et al. (2013) reported that the phytic acid content decreased in brown rice as the degree of milling, as defined by the

machining accuracy of the milling equipment, increased [20]. Shobana et al. (2011) evaluated the changes in content of phytochemicals, dietary fiber, and γ-Oryzanol in different fractions according to the degree of milling in two Indian rice varieties [21]. There have been fewer studies on other compounds including carotenoids (lutein, zeaxanthin, β-cryptoxanthin, and β-carotene), and phenolics. Phenolics include phenolic acids (p-coumaric, caffeic, ferulic, vanillic, and syringic acids) and flavonoids (flavonols, flavones, catechins, and anthocyanins). Some studies have focused on the total phenolic contents from the outer to the inner layers [22]. In recent years, others have compared the total phenolic contents in the rice bran, rice bran layer (rice bran except embryo) and rice embryo [23]. Overall, these studies suggest that the beneficial phytochemicals in whole grain rice are distributed in the free, soluble-conjugated and bound forms in inner and outer layers of whole brown rice. However, the rice samples were of different species, thus complicating the direct comparison of data on these four fractions. Finally, most of the previous studies have measured the changes of nutrients at different milling times using the rice machining accuracy as an index, which does not correspond to the four different fractions of brown rice. Because of this, information on the beneficial phytochemicals of the different fractions of *indica* and *japonica* rice is of great importance to researchers. Therefore, studying the phytochemicals of these different fractions has important scientific significance and economic value for guiding the lighted degree to which brown rice and embryo rice should be processed.

The overall objective of the present study is to provide information which

quantifies the content of these antioxidant phytochemicals in the different fractions of whole brown rice, to satisfy the needs of food producers and rice consumers. The specific objectives of this study were: (1) to reveal the distribution and difference of phenolic contents and antioxidant activity in four tissue rice fractions—pericarp, aleurone layer, embryo and endosperm; (2) to demonstrate the differences of ratios of free and bound phenolics/antioxidant activity to total; and（3）to determine the percentage contributions of the different fractions to the total phenolic contents and antioxidant activity of whole brown rice.

2. Materials and methods

2.1. Chemicals and reagents.

2′,7′-Dichlorofluorescin diacetate (DCFH-DA), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 2,2′-Azobis-(2-amidinopropane) dihydrochloride (ABAP), 3′,6′-Dihydroxyspiro[isobenzofuran-1(^{3}H), 9′-(^{9}H)-xanthene]-3-one disodium salt and quercetin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gallic, protocatechuic, chlorogenic, hydroxybenzoic, vanillic, caffeic, syringic, coumaric, ferulic, syringic and isoferulic acids, catechin and epicatechin were purchased from Aladdin Reagents (Shanghai, China). High-performance liquid chromatography-grade acetic acid and acetonitrile were obtained from Fisher (Suwanee, GA, USA). HepG2 human liver cancer cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Williams' Medium E and Hanks' Balanced Salt Solution (HBSS) were purchased from Gibco Life Technologies

(Grand Island, NY, USA). Fetal bovine serum was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). All other chemicals used were of analytical grade or above.

2.2. Grain Samples and Sample Preparation.

Grains of the *indica* cultivar Zaomiao and the *japonica* cultivar Wujingyun 27, which are consumed primarily in Southern and Northern China, respectively, were obtained from the Experimental Farm of the Rice Research Institute of Guangdong Academy of Agricultural Sciences in 2013. They were sown in late March 2013 and harvested in mid-July. The rice grains were air-dried until their moisture content was reduced to approximately 13% and stored at room temperature for three months. These rice samples were milled to separate the husk from the brown rice. The husk was not included in the analysis. The embryos were separated manually from the brown rice sample (w_0, ~5 g) and weighed (w_1). The degermed brown rice samples were successively polished to collect different tissue fractions (pericarp, aleurone layer and endosperm) of the brown rice samples using a Satake mill (Satake Corp., Tokyo, Japan). The weights of the pericarp and aleurone layer were sequentially marked as w_2, w_3. The brown rice samples, divided into four tissue fractions, were ground separately to a powder able to pass through a 60-screen mesh, then stored at −20 °C until further analysis. The percentages of pericarp, aleurone layer, embryo and endosperm were calculated using the following equations [24]:

Pericarp (%) = $w_2 / w_0 \times 100$

Aleurone layer (%) = $w_3 / w_0 \times 100$

Endosperm (%) = $(w_0 - w_1 - w_2 - w_3) / w_0 \times 100$

Embryo (%) = $w_1 / w_0 \times 100$

2.3. Extraction of phenolic compounds

2.3.1. Extraction of free phenolic compounds.

The method was adapted from Zhang, Zhang, Zhang & Liu (2010)[25-27] with a few modifications. First, 0.5 g pericarp, 0.5 g aleurone layer, 0.5 g embryo and 2 g endosperm were weighed precisely and transferred into a 100 mL centrifuge tube. 50 mL 80% acetone (v/v) pre-cooled at 5 °C for 20 min was then added. The mixture was homogenized for 5 min using a homogenizer at 10,000 rpm and centrifuged at 2500 g for an additional 10 min. The supernatant was then collected. 50 mL 80% acetone was added to the precipitate and the extraction procedure above was repeated. The supernatants obtained from the two centrifugations were pooled and rotary-evaporated at 45 °C. The residue was dissolved in 10 mL methanol to give the free phenolic extract solution. The solution was then split and stored at −20 °C. The weighing and extraction were performed in triplicate.

2.3.2. Extraction of bound phenolic compounds.

The method was adapted from Adom, Sorrells and Liu (2003) [28] with a few modifications. 40 mL 2M NaOH solution was added to the precipitate that had undergone the free phenolic extraction process described in Section 2.3.1. The solution obtained was then protected by nitrogen and shaken at room temperature for 1 h. After adjustment to pH 1 using 6 mol/L HCl solution, the solution was extracted and degreased with 100 mL n-hexane and then extracted five times with ethyl acetate.

All the ethyl acetate extract phases were pooled and rotary-evaporated to dryness at 45 °C. The residue was dissolved in 10 mL methanol to give the bound phenolic extract solution, which was then stored at −20 °C. All the procedures were performed in triplicate.

2.3.3. Determination of total phenolic content.

The method was adapted from Dewanto, Wu, Adom and Liu (2002) [29] with a few modifications. 0.125 mL of the free/bound phenolic extract solution was pipetted and added to 0.5 mL distilled water and 0.5 mL Folin's phenol reagent. After mixing well, the solution was allowed to react for 6 min at 25 °C and then 1.25 mL 7% (m/v) Na_2CO_3 solution and 1.25 mL distilled water were added. After mixing well, this solution was kept at 25 °C in the dark for 90 min. The absorbance was then determined at a wavelength of 760 nm. The blank control sample was prepared by substituting the sample extract solutions with 0.125 mL methanol. The standard curve was plotted with gallic acid as the standard. The total phenolic content was determined as mg gallic acid equivalents per 100 g dry weight (mg GAE /100 g DW). The determinations above were performed in triplicate.

2.4. Determination of total flavonoid content.

The determination method for total flavonoid content was adapted from Dewanto et al. (2002) [29] with a few modifications. 0.3 mL free/bound phenolic extract solution was pipetted and then 1.5 mL distilled water and 0.09 mL 5% (m/v) $NaNO_2$ solution were added. After thorough mixing, the solution was allowed to react for 6 min at 25 °C and then 0.18 mL 10% (m/v) $AlCl_3 \cdot 6H_2O$ was added. After reacting at 25 °C

for a further 5 min, 0.6 mL 1 mol/L NaOH solution was added and made up to 3 mL with distilled water. The absorbance of the solution was determined at a wavelength of 510 nm. The blank control sample was prepared by substituting the extract solution with 0.3 mL methanol. The standard curve was plotted using catechin as the standard. The total flavonoid content was determined as mg catechin equivalents per 100 g dry weight (mg CE /100 g DW). The determinations above were performed in triplicate.

2.5. Determination of phenolic composition.

The phenolic composition was determined using an Agilent 1200 high performance liquid chromatograph (Waldbronn, Germany) equipped with a VWD ultraviolet detector. The chromatographic conditions were: Zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm) (Agilent, Palo Alto, CA, USA); mobile phase A: acetonitrile, B: 0.4% glacial acetic acid; flow rate: 1.0 mL/min; column temperature: 30 °C; detection wavelength: 280 nm; gradient elution procedure: 0–40 min, A 5–25%; 40–45 min, A 25–35%; 45–50 min, A 35–50%. The total run time was 50 min and the sample volume was 20 μL. The recovery test, performed using the standards, showed over 96–99% recovery, meeting the requirement of quantitative analyses. The determination, performed in triplicate, was performed by comparing the retention times of the samples with the standards.

2.6. Measurement of oxygen radical scavenging capacity (ORAC).

The method was adapted from Zhang et al. (2010) [25] with a few modifications. The free/bound phenolic extract solution from the brown rice was dried by nitrogen flow and then diluted with 75 mmol/L phosphate buffer so that the total phenolic content

was controlled within a certain range. The dilution of the standards and dissolution of samples all used 75 mmol/L potassium phosphate buffer (pH 7.4). 20 μL buffer solution (blank), 20 μL Trolox standard solutions, 20 μL phenolic extract solution and 200 μL 0.96 μmol/L fluorescein working solution were added to the wells of a 96-well plate The plate was then incubated at 37 ℃ for 10 min. Next, 20 μL freshly prepared 119 mmol/L ABAP solution was added quickly to each well using a multichannel pipettor. The multi-functional microplate reader was immediately launched to detect the fluorescence intensity of each well continuously to monitor the fluorescence decay (37 ℃, excitation wavelength 458 nm, emission wavelength 538 nm). The detection was performed for 35 cycles (4.5 min each cycle). The total oxygen radical absorbance capacity (ORAC) index value was determined as Trolox equivalent per gram dry weight (μmol TE /g DW). All procedures were performed in triplicate.

2.7. Measurement of cellular antioxidant activity.

The cellular antioxidant activity (CAA) test was conducted using the method of Wolfe and Liu (2007) [30]. Briefly, human hepatoma cells (HepG2 cells) were inoculated onto the 96-well plate at a cell density of 6×10^4 for the 100 μL culture solution (DMEM containing 10% fetal calf serum) in each well. Each inoculated well was rinsed with PBS. Then, 10 μL free/bound phenols extract solution (containing 25 μmol·L-1 DCFH-DA) was added to each well and incubated at 37 °C under a 5% CO_2 atmosphere for 1 h. The plate was then taken out and 100 μL HBSS culture medium (containing 600 μmol/L ABAP) were added to each well, except for the blank well

where 100 μL HBSS culture medium without ABAP were added. The plate was then put into a fluorescence microplate for scanning where the fluorescence values of all wells were detected continuously (at 37 ℃, excitation wavelength 485 nm, emission wavelength 538 nm) for 12 cycles (each cycle 5 min). The calculation formula used for CAA was:

$$\text{CAA (unit)} = 1 - (\int SA / \int CA)$$

where, ∫SA and ∫CA are the integral areas under the sample time-fluorescence value and control time-fluorescence value curves, respectively. The median effective concentrations (EC50) of the sample polyphenol extracts were calculated according to the median effect principle of log (fa/fu) vs. log (dose), where fa represents the samples' effects of actions (CAA unit) and fu represents 1 − CAA unit. The calculation of EC50 was based on three parallel tests then converted into CAA values as μmol quercetin equivalents /100 g dry weight (μmol QE /100 g DW). All the procedures above were performed in triplicate.

2.8. Statistical Analysis.

The data analysis and plotting were performed using SPSS 13.0 (SPSS Inc. Chicago, IL, USA). The data were provided in the form of means ± SD. One-way analysis of variance was used to compare the mean values of phenolic content and antioxidant capacity of the different fractions of brown rice using the LSD method at a significance level of $p < 0.05$.

3. Results

3.1. Weight of pericarp, aleurone layer, embryo and endosperm

The percentages of pericarp, aleurone layer, embryo and endosperm determined are 2.0±0.1, 4.7±0.4, 2.5±0.4, 90.8±0.8 of *japonica* rice and 2.0±0.2, 4.7±0.5, 2.7±0.8, 90.6±1.2 of *indica* rice. The average percentages of pericarp, aleurone layer, embryo and endosperm fractions of brown rice were 2.0, 4.7, 2.6 and 90.7, respectively. These measurements were only for research purposes and not involved in the milling process.

3.2. Total phenolic content.

Table 1 provides the free, bound and total phenolic contents of the four tissue fractions of the two types of brown rice.

There were significant differences in the free, bound and total phenolic contents between the four tissue fractions of *japonica* and *indica* brown rice ($p < 0.05$). For japonica rice, the total phenolic contents were highest in the pericarp ($p < 0.05$), followed by aleurone layer, embryo. The total phenolic contents were lowest in the endosperm ($p < 0.05$). The order of the four tissue fractions of *indica* brown rice was very similar with the ranking of *japonica* brown rice.

Comparing *indica* and *japonica* rice, the distribution of free, bound and total phenolic contents among the four fractions was very similar, However, the values of free, bound and total phenolic contents of *japonica* compared with *indica* were 48.0%, 94.5% and 65.5% higher ($p < 0.05$), respectively in the pericarp, 46.4%, 77.6% and 57.6% higher ($p < 0.05$), respectively in the aleurone layer, 105.4%, 56.7% and 88.3% higher ($p < 0.05$), respectively in the embryo and 2.4%, 12.2% and 5.6% higher ($p <$

0.05), respectively in the endosperm. This may have been due to the genetic differences between the subspecies or types.

3.3. Total flavonoid content.

Table 1 shows the free, bound and total flavonoid contents in the four fractions of the two types of brown rice.

There were significant differences in the free, bound and total flavonoid contents between the four fractions of *japonica* and *indica* brown rice ($p < 0.05$). The trend of the flavonoids of two types of brown rice was the same with their phenolics.

Comparing *indica* and *japonica* rice, there was a similar distribution of free, bound and total phenolic contents among the four fractions. The values of bound and total flavonoid contents of *indica* compared with *japonica* were 64.0% and 20.1% higher ($p < 0.05$), respectively in the pericarp and 54.8% and 23.7% higher ($p < 0.05$), respectively in the aleurone layer. The genetic differences between these subspecies or types may have led to these differences. The values of free, bound and total flavonoid contents were more or less similar in both the embryo and endosperm fractions for the two types.

3.4. Phenolic composition.

Table 2 shows the individual phenolic composition and contents of four successive fractions in the two types of brown rice. There were significant differences in free and bound phenolic contents among the four fractions in the two types of brown rice ($p < 0.05$). The composition and the forms of phenolic compounds present were similar for the two types of brown rice. Ferulic and coumaric acids were predominant in brown

rice, both existing mainly in the bound form. Of the phenolics, ferulic acid had the highest content and was richest in the pericarp (mean value = 2204.7 μg /g DW). Coumaric acid was also richest in the pericarp (mean value = 944.7 μg /g DW). These results suggest that phenolic acids are concentrated mainly in the pericarp fraction of whole brown rice. The much smaller amounts of epicatechin detected in the embryo existed in the free form. The epicatechin content in the embryo (mean value = 535.9 μg/g DW) was significantly higher than in the other fractions. Gallic, protocatechuic, hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic and isoferulic acids, catechin and quercetin were detected at low or trace levels in all extracts of the identified phenolic acids.

3.5. Antioxidant capacity

3.5.1. ORAC

Table 3 shows the free and bound ORAC values in four fractions of the two types of brown rice.

There were significant differences in free, bound and total ORAC values among the four fractions of *japonica* and *indica* brown rice ($p < 0.05$). In both types of brown rice, similar with phenolics, the total ORAC values were highest in the pericarp ($p < 0.05$) and lowest in the endosperm ($p < 0.05$). The sequence of total ORAC values was: pericarp > aleurone layer > embryo > endosperm.

Comparing *indica* and *japonica* rice, the distribution of free, bound and total ORAC values among the four fractions was similar. However, the free, bound and total ORAC values of *japonica* were 18.2%, 25.1% and 21.6% higher ($p < 0.05$) than

those of *indica*, respectively in the pericarp and 23.1%, 25.9% and 24.4% higher ($p < 0.05$) than those of *indica*, respectively in the aleurone layer. These differences may have been caused by the genetic differences between the subspecies or types. The free, bound and total ORAC values in both the embryo and endosperm fractions of *japonica* were a little higher or very similar to those of *indica*.

3.5.2. CAA

Table 4 shows the free and bound CAA values in the four fractions of the two types of brown rice.

There were significant differences in the free, bound and total CAA values among the four fractions of *japonica* and *indica* brown rice ($p < 0.05$). For japonica rice, the total CAA values were highest in the pericarp ($p < 0.05$) and lowest in the endosperm ($p < 0.05$). The sequence of total CAA values was: pericarp > aleurone layer > embryo > endosperm. Whereas, the sequence of total CAA values of indica rice was aleurone layer > embryo > pericarp > endosperm. The discrepancy between ORAC and CAA activity of these compounds could be at least partly attributed to their difference in chemical structure, which affects their ability to scavenge free radicals and the level of cellular absorption and metabolism.

Comparing *indica* and *japonica* rice, a similar distribution of free, bound and total CAA values among the four fractions was observed. The values of free, bound and total CAA values in the pericarp of *japonica* were 26.2%, 31.1% and 29.7% higher ($p < 0.05$) than those of *indica*, respectively, while the values of free, bound and total CAA in the aleurone layer of *indica* were 29.3%, 10.5% and 14.2% higher ($p < 0.05$)

than those of *japonica*, respectively, and in the embryo, 28.5%, 164.7% and 51.4% higher ($p < 0.05$) than those of *japonica*, respectively. The genetic differences between these subspecies or types may account for these differences. The free, bound and total CAA values in the endosperm fraction were almost the same in the two types of rice.

4. Discussion

4.1. Phenolic contents of four tissue fractions of whole brown rice

Brown rice is botanically defined as the fruit of the rice plant, but its seed is entirely covered with a thin pericarp. The testa that covers the seed is also very thin, inside of which are the aleurone layer, the embryo and the endosperm [31]. The distribution of phenolics has not been examined in the four tissue fractions of brown rice. At the tissue level, higher concentrations of phenolic compounds are found in the outer layers of plants, e.g., in the epidermis, than in the inner layers [32]. Previous study indicated that the concentrations of phenolic acids decreased from the aleurone layer to endosperm in brown rice [33]. Another also found that bran and embryo exhibit higher free and bound phenolic content than endosperm [24]. The results of the present study provide a more comprehensive understanding of the distribution of antioxidants in four tissue fractions of brown rice, pericarp, aleurone layer, embryo and endosperm. Analysis of the free and bound phenolic contents in these tissue fractions suggested that it was highest in the pericarp and lowest in the endosperm. The phenolic content tended to decrease progressively from the outside to the center of brown rice, in a

similar way to other nutrients [16, 34]. These results have demonstrated that the concentration of phenolics varies in the different fractions of brown rice, suggesting the potential for their greater use as different sources of concentrated antioxidants from natural whole brown rice.

Phenolic acids and flavonoids are important polyphenols in plants. The results have shown that the phytochemicals (phenolics and flavonoids) in these four tissue fractions (pericarp, aleurone layer, embryo and endosperm) existed mainly in the free and bound forms. The bound form in the pericarp, aleurone layer, embryo and the endosperm provided, on average, 41.8%, 38.5%, 31.2% and 33.8%, respectively of phenolics and 36.2%, 38.4%, 27.1% and 34.9%, respectively of flavonoids (sum of bound values of two types of rice/sum of total values of two types of rice). Liu (2007) has shown that bound phenolics cannot be decomposed by human digestive enzymes; after the phenolics reach the colon, the ester bond and the macromolecules on the cell wall will be destroyed during fermentation by microbial flora, thus releasing the phenolics, which provide a constant source for humans [35]. The data in the present study has shown that the pericarp and aleurone layer fractions contributed more bound phenolics than the embryo and endosperm fractions. This indicated that they may deliver a higher level of phenolics to the colon and hence healthy effects because of these higher proportions of bound phenolics. One study has demonstrated that the bound phenolic content of whole brown rice grain contributed more than 60% to the total [28]. Another reported that the bound fraction provided 12.2% of phenolics and 29.3% of flavonoids in rice bran and 26.7% of phenolics and 40.7% of flavonoids in

polished rice [10]. The different levels of bound phenolics are primarily not only attributable to the various phenolic acids in the different tissue fractions but also to the rice types. Phenolic acids in plants are not uniformly distributed at either the tissue or cellular levels. At the same tissue level, higher concentrations of phenolic acids are found in the outer layers of plants than in the inner layers [24, 32]. The data in the present study have comprehensively documented the distribution of free and bound phytochemicals (phenolic and flavonoids) content in the four tissue fractions of whole brown rice.

In whole brown rice, the pericarp, aleurone layer, and embryo fraction contribute 13.0%, 28.5% and 8.8% to the total phenolics and 14.1%, 29.7% and 9.1% to the total flavonoids, respectively. The endosperm fraction contributes the remaining 49.7% to the total phenolic and 47.1% to the total flavonoids (Fig. 1a & b). Thus, whole brown rice is more abundant in phenolic resources than the endosperm fraction, which agrees with Zhou, Robards, Helliwell and Blanchard (2004) who reported that phenolics were distributed mainly in the rice bran [36]. This indicates that brown rice is a good dietary source of antioxidants when compared with the polished rice/endosperm generally consumed in the human diet. Importantly, our results have shown that in the rice bran fraction, the aleurone layer contributed most of the phenolics and flavonoids, followed by the embryo and endosperm fractions. Therefore, this present analysis of the contributions of the four tissue fractions of whole brown rice has provided data that can lead to improving its application, such as whole brown rice may be further processed into bran, aleurone layer and embryo rice and used in food products.

These food products may have different requirements regarding sensory properties, quality and different health benefits for different groups of consumers. In the past, because the distribution of phenolics in the different fractions of brown rice had not been clear, the consumption of whole brown rice had been overemphasized to consumers, so its further promotion to consumers should be re-evaluated. In fact, cooking embryo rice is popular because of its taste, and because it retains some nutrients from the embryo and part of the aleurone layer. The results of the present study provide the necessary information for evaluating the health benefits from consuming embryo rice. These data also provide help for the judicious control of the degree of milling during the processing of whole brown rice with regard to its sensory quality and phenolics content. Generally, brown rice milled to a higher degree has a better appearance, but the phytochemicals, beneficial for human health, are discarded [2]. The study presented the percentage data of phenolics, flavonoids and antioxidant activity in different rice forms, including semi-brown rice, embryo rice, lightly milled rice and polished rice in the market to the whole brown rice. Therefore, the present study has provided knowledge for encouraging the consumption of semi-brown rice, embryo rice, lightly milled rice rather than brown rice or polished rice (Fig. 2).

4.2. Phenolic components of four tissue fractions of brown rice

The distribution of phenolic acids in brown rice was not uniform, being more concentrated in the bran layer and less in the endosperm [33, 37]. Recent studies have concentrated on phenolics in brown rice or the rice bran layers of whole brown rice. Previous study found that seven different free and bound phenolic acids (gallic,

440 protocatechuic, caffeic, clove, chlorogenic, coumaric and ferulic acids) existed mainly
441 in the rice bran fractions with most present in the bound form [10]. The present results
442 were consistent with this conclusion that the phenolic acids were mainly present in the
443 rice bran fraction. Zhou et al. (2004) found that brown rice contained more phenolic
444 acids (ferulic, coumaric, gallic, vanillic, caffeic and syringic acids) than polished rice
445 [36]. The present study has shown that the coumaric acid contents in the pericarp,
446 aleurone layer and embryo were 59, 39 and 17 times higher, respectively than in the
447 endosperm, while the ferulic acid contents were 19, 17 and 8 times higher,
448 respectively. The main active phenolic acids were coumaric and ferulic in rice bran,
449 which mainly connect to some sugar residues or side chains of xylan polysaccharide
450 in the cell wall through ester linkages [34]. These phenolic acids are also commonly
451 present in the bound form and as components of complex structures. Overall, the
452 significant differences in the distribution of these phenolic acids between the pericarp,
453 aleurone layer, embryo and endosperm fractions have now been defined.

454 In the present study, flavonoids such as catechin, epicatechin and quercetin were
455 detected in different parts of whole brown rice, an aspect previously unreported. The
456 class of flavonoids containing (−)-epicatechin, (+)-catechin and quercetin are
457 widespread in fruits and whole grains. The present study detected them in both the
458 free and bound form, another aspect previously unreported. In the pericarp, aleurone
459 layer and embryo fractions, the content of (−)-epicatechin was relatively higher with
460 average values of 132.6, 90.9 and 595.4 μg /g, respectively, and of quercetin with
461 average values of 33.9, 28.6 and 20.4 μg /g, respectively. They were not detected in

462 the endosperm fraction. The content of (+)-catechin was found to be low or not
463 detected in the pericarp, aleurone layer, embryo and endosperm fractions. It should be
464 noted that in the present study these individual flavonoid compounds have been
465 analyzed by a direct comparison with corresponding standards based on retention
466 times. Therefore, this tentative identification method needs further confirmation using
467 other methods such as HPLC-MS to give a positive identification.

468 *4.3. Antioxidant activity of four tissue fractions of whole brown rice*

469 Four different fractions of brown rice have been surveyed using both the ORAC
470 and the CAA assays. The ORAC assay is a traditional chemical method based on the
471 hydrogen atom transfer reaction mechanism. The CAA assay is an improvement over
472 the traditional chemistry-based antioxidant activity assay. It provides a better
473 prediction of antioxidant behavior in biological systems as it takes into account some
474 aspects of cell uptake, metabolism and distribution of bioactive compounds [26-27, 38].
475 The results of the present study have shown that the four different fractions of whole
476 brown rice exhibited a wide range of antioxidant potentials. Although the range of
477 ORAC and CAA mean values did not vary greatly, the total antioxidant activity
478 showed statistically significant differences between them ($p < 0.05$).

479 Phenolic compounds contribute to antioxidant activity but reports examining the
480 contributions of free and bound antioxidant activity in the pericarp, aleurone layer,
481 embryo and endosperm fractions are limited. In the present study, the results of total
482 ORAC values agreed with those of Ti et al. (2014), who determined them in the rice
483 bran and milled rice fractions of five *indica* rice varieties. The ORAC values ranged

484 from 182.2–221.2 μmol TE /g DW in rice bran and from 16.1–24.5 μmol TE /g DW in
485 milled rice with the antioxidant activity mainly distributed in the free form. It is
486 difficult to compare the CAA values as no others have been reported for brown rice.
487 However, the present study has shown that the CAA values in the pericarp, aleurone
488 layer and endosperm existed mainly in the bound form, while in the embryo it existed
489 mainly in the free form. This result disagreed with previous articles that claimed that
490 free phenolics formed the majority of antioxidants in brown rice. Such a difference
491 might be because of differences in the rice varieties and growing environments. The
492 composition of free and bound individual phenolics was also different with significant
493 differences in CAA values between the individual phenolics. Finally, differences in
494 solubility, molecular size and polarity of the wide variety of compounds present in
495 grains, fruits and vegetables give them unique bioactivity and distribution at the
496 cellular, organ and tissue levels [30]. Thus different phenolic compounds showed
497 significant differences in CAA antioxidant activity.

498 As mentioned for whole brown rice (Fig. 1c & d), the total ORAC and CAA values
499 distributed in the pericarp, aleurone layer, embryo and endosperm fractions,
500 respectively were 18.2%, 38.0%, 11.1% and 32.8% for ORAC, and 14.6%, 38.0%,
501 16.9% and 30.5% for CAA. The endosperm fraction, the whitest portion of the grain,
502 is generally favored because of its better taste and appearance. The present data has
503 indicated that although the endosperm fraction was larger, proportionately, than the
504 other fractions of brown rice, its antioxidant activity was not highest. Previous study
505 has reported that the rice bran/embryo fraction had a higher antioxidant activity than

506 the endosperm fraction [10], which was basically consistent with the results of the
507 present research. However, the antioxidant activity contribution of each fraction to
508 brown rice after actual processing was previously unclear because of the complex
509 structure of rice bran. The present research has found that, although only forming a
510 small proportion of whole brown rice, the aleurone layer was the largest contributor of
511 antioxidant activity while the endosperm was ranked second although forming a larger
512 proportion of whole brown rice. The pericarp and embryo fractions contributed nearly
513 30% of antioxidant activity, but consist of only 4.6% of the weight of whole brown
514 rice. The present study has provided information on the contribution of four
515 successive fractions to the free, bound and total antioxidant activities of brown rice
516 using two assays. This information is necessary for processing whole brown rice and
517 its products for the food and pharmaceutical markets. The present research is part of
518 ongoing efforts to promote added value to the production and use of brown rice for
519 preventing human chronic diseases related to oxidative stress.
520

521 **5. Conclusions**

522 To summarize, this study has shown that the contents of free and bound phenolics
523 and antioxidant activity in four tissue fractions were significantly different. The
524 highest phenolic content and antioxidant activity were in the pericarp fraction,
525 whereas those in the endosperm fraction were lower. The phenolics contents and
526 antioxidant activity in the pericarp, aleurone layer, embryo and endosperm in *indica*
527 and *japonica* rice were present both in the free and bound forms. The aleurone layer

fraction contributed a larger or similar total phenolics content, composition and antioxidant activity compared with the endosperm fraction although it formed a smaller proportion of whole brown rice. Thirteen phenolic compounds (gallic, protocatechuic, hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, isoferulic, coumaric and ferulic acids; catechin, epicatechin, quercetin) were detected in the four tissue fractions, with ferulic acid at the highest level followed by coumaric acid. Measuring the antioxidant activity of grains using cell culture is an important step in screening for potential bioactivity and is biologically more representative than data obtained from chemistry-based antioxidant activity assays. The present study used ORAC and CAA assays to confirm significant antioxidant effects of different fractions of brown rice. Therefore, the research will have significant value for deciding the type of rice processing, including different tissue fractions and for guidance on the consumption of whole brown rice.

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References

1. FAOSTAT (2009). FAO Statistics Division 2009. <http://faostat.fao.org/>. 18 September 2009.
2. L. Lamberts, E. De Bie, G. E. Vandeputte, W. S. Veraverbeke, V. Derycke, W. De Man, and J. A. Delcour, Effect of milling on colour and nutritional properties of rice. *Food Chem,* 2007, **100,** 1496–1503.
3. M. W. Zhang, *Special rice and its processing technology*. 2000.
4. L. Liu, J. J. Guo, R, F. Zhang, Z. C. Wei, Y. Y. Deng, J. X. Guo, and M. W. Zhang. Effect of degree of milling on phenolic profiles and cellular antioxidant activity of whole brown rice. *Food Chem,* 2015, **100,** 1496–1503.
5. O. Okarter, and R. H. Liu, Health benefits of whole grain phytochemicals. *Crit Rev Food Sci Nutr,* 2010, **50,** 193–208.
6. Benavente-Garcia, O., Castillo, J., Marin, F. R., Ortuno, A., & Del Rio, J. A.. Uses and properties of citrus flavonoids. *J Agric Food Chem*, 1997, **45,** 4505–4515.
7. Manach, C., Mazur, A., & Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol*, 2005, **16,** 77–84.
8. Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol Rev*, 52, 673–751.
9. Samman, S., Lyons Wall, P. M., & Cook, N. C. *Flavonoids and coronary heart disease: Dietary perspectives.* 1998, pp. 469–482.
10. H. H. Ti, Q. Li, R. F. Zhang, M. W. Zhang, Y. Y. Deng, Z. C. Wei, J. W. Chi, and Y. Zhang, Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in Southern China. *Food Chem,* 2014, **159,** 166–174.

11. Liu, K. L., & Gu, Z. X.. Selenium accumulation in different brown rice cultivars and its distribution in fractions. *J Agric Food Chem*, 2009, **57,** 695–700.

12. A. Abdul-Hamid, R. R. Raja Sulaiman, A. Osman, and N. Saari, Preliminary study of the chemical composition of rice milling fractions stabilized by microwave heating. *J Food Compos Anal,* 2007, **20,** 627–637.

13. J. K. Park, S. S. Kim, and K. O. Kim, Effect of milling ratio on sensory properties of cooked rice and on physicochemical properties of milled and cooked rice. *Cereal Chem,* 2001, **78,** 151–156.

14. A. P. Resurreccion, B. O. Juliano, and Y. Tanaka, Nutrient content and distribution in milling fractions of rice grain. *J Agric Food Chem*, 1979, **30,** 475–481.

15. N. Singh, H. Singh, K. Kaur, and B. M. Singh, Relationship between the degree of milling, ash distribution pattern and conductivity in brown rice. *Food Chem,* 2000, **69,** 147–151.

16. T. Itani, M. Tamaki, E. Arai, and T. Horino, Distribution of amylose, nitrogen, and minerals in rice kernels with various characters. *J Agric Food Chem*, 2002, **50,** 5326–5332.

17. C. Prom-u-thai, C. Sanchai, B. Rerkasem, S. Jamjod, S. Fukai, I. D. Godwin, and L. Huang, Effect of grain morphology on degree of milling and iron loss in rice. *Cereal Chem,* 2007, 84, 384–388.

18. J. Liang, Z. Li, K. Tsuji, K. Nakano, M. J. R. Nout, and R. J. Hamer, Milling characteristics and distribution of phytic acid and zinc in long-, medium- and short-grain rice. *J Cereal Sci,* 2008, **48,** 83–91.

19. S. Yu, Z. T. Nehus, T. M. Badger, and N. Fang, Quantification of vitamin E and γ-oryzanol components in rice germ and bran. *J Agric Food Chem*, 2007, **55,** 7309–7313.

20. J. L. F. Monks, N. L.Vanier, J. Casaril, R. M. Berto, M. De Oliveira, C. B. Gomes, M. P. De Carvalho, A. R. G. Dias, and M. C. Elias, Effects of milling on proximate composition, folic acid, fatty acids and technological properties of rice. *J Food Compos Anal,* 2013, **30,** 73–79.

21. S. Shobana, N. Malleshi, V. Sudha, D. Spiegelman, B. Hong, F. Hu, W. Willett, K. Krishnaswamy, and V. Mohan, Nutritional and sensory profile of two Indian rice varieties with different degrees of polishing. *Int J Food Sci Nutr,* 2011, **62,** 800–810.

22. S. Jang, and Z. M. Xu, Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. *J Agric Food Chem*, 2009, **57,** 858–862.

23. A. Moongngarm, N. Daomukda, and S. Khumpika, Chemical Compositions, Phytochemicals, and Antioxidant Capacity of Rice Bran Layer, and Rice Germ. *APCBEE Procedia*, 2012, 2, 73–79.

24. Y. F. Shao, F. F. Xu, X. Sun, J. S. Bao, and T. Beta, Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). *J Cereal Sci,* 2014, **59,** 211-218.

25. M. W. Zhang, R. F. Zhang, F. X. Zhang, and R. H. Liu, Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J Agric Food Chem*, 2010, **58,** 7580–7587.

26. W. Song, C. M. Derito, M. K. Liu, X. J. He, M. Done, and R. H. Liu, Cellular Antioxidant Activity of Common Vegetables. *J Agric Food Chem,* 2010, **58,** 6621–6629.

27. K. L. Wolfe, X. M. Kang, X. J. He, M. Done, Q. Y. Zhang, and R. H. Liu, Cellular Antioxidant Activity of Common Fruits. *J Agric Food Chem.* 2008, **56,** 8418–8426.

628 28. K. K. Adom, M. E. Sorrells, & R. H. Liu, Phytochemical profiles and antioxidant
629 activity of wheat varieties. *J Agric Food Chem*, 2003, **51***,* 7825–7834.

630 29. V. Dewanto, X. Wu, K. K. Adom, and R. H. Liu, Thermal processing enhances
631 the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric*
632 *Food Chem*, 2002, **50,** 3010–3014.

633 30. K. A. Wolfe, and R. H. Liu, Cellular antioxidant activity [CAA] assay for
634 assessing antioxidants, foods, and dietary supplements, *J Agric Food Chem*, 2007,
635 **55,** 8896–8907.

636 31. K. Hoshikawa, Science of the Rice Plant, 1993, pp. 377–380.

637 32. M. Naczk, and F. Shahidi, Extraction and analysis of phenolics in food. *J*
638 *Chromatogr A,* 2004, **1054,** 95–111.

639 33. S. Butsat, and S. Siriamornpun, Antioxidant capacities and phenolic compounds
640 of the husk, bran and endosperm of Thai rice. *Food Chem,* 2010, **119,** 606–613.

641 34. J. I. Wadsworth, Rice: Science and technology, 1994, pp. 139–176.

642 35. R. H. Liu, Whole grain phytochemicals and health. *J Cereal Sci,* 2007, **46,**
643 207–219.

644 36. Z. Zhou, K. Robards, S. Helliwell, and C. Blanchard, The distribution of phenolic
645 acids in rice. *Food Chem,* 2004, 87, 401–406.

646 37. M. Naczk, and F. Shahidi, The effect of methanol-ammonia-water treatment on
647 the content of phenolic acids of canola. *Food Chem,* 1989, 31, 159–164.

648 38. J. Sun, Y. F. Chu, X. Wu, and R. H. Liu, Antioxidant and antiproliferative
649 activities of common fruits. *J Agric Food Chem*, 2002, **50,** 7449–7454.

650

Table 1 Total phenolic and flavonoid content of four fractions in two types (subspecies) of whole brown rice.

Rice type	Tissue fraction	Free	Bound	Total
Phenolics[a]				
Japonica	Pericarp	484.4±5.2a[#](55.7) [##]	385.1±3.1a(44.3)	869.5±4.3a
	Aleurone layer	473.3±9.0a(59.7)	319.5±8.6b(40.3)	792.8±6.3b
	Embryo	351.6±5.8b(70.9)	144.5±3.4c(29.1)	496.1±2.7c
	Endosperm	38.1±1.3c(65.3)	20.3±0.6d(34.7)	58.4±1.9d
Indica	Pericarp	327.4±2.2a*(62.3)	198.0±3.2a *(37.7)	525.5±1.2a*
	Aleurone layer	323.3±4.2a*(64.2)	179.9±6b*(35.8)	503.2±8.6b*
	Embryo	171.2±3.1b*(65)	92.2±2c*(35)	263.4±4.3c*
	Endosperm	37.2±0.6c(67.2)	18.1±0.5d(32.8)	55.3±0.3d
Flavonoids[b]				
Japonica	Pericarp	457.6±9.8a (69.8)	198.0±3.2a (30.2)	655.6±8.2a
	Aleurone layer	383.5±15.1b (66.3)	194.8±6.2a (33.7)	578.4±15.6b
	Embryo	299.7±12.2c (76.5)	92.2±2.0b (23.5)	391.9±13.6c
	Endosperm	33.8±2.1d (63)	19.8±2.8c (37)	53.6±1.9d
Indica	Pericarp	462.7±4.4a * (58.8)	324.8±4.5a*(41.2)	787.5±5.4a*
	Aleurone layer	413.7±1.4b * (57.8)	301.6±15.8b*(42.2)	715.3±15.7b*
	Embryo	239.7±0.5c* (68.8)	108.5±3.2c* (31.2)	348.1±3.0c*
	Endosperm	35.0±2.3d (67.3)	17.0±2.0d (32.7)	52.0±1.1d

Values with no letters in common in each column are significantly different (p<0.05)

Values in parentheses indicate percentage contribution to the total phenolics.

* Values of *Japonica* and *Indica* rice in each fraction are significantly different (p<0.05)

[a] mg GAE/100 g DW. [b] mg CE/100 g DW.

Table 2 Phenolic composition of four fractions in two types of whole brown rice.

Rice type	Tissue fraction	Free (μg/ g)	Bound (μg/ g)	Total (μg/ g)
Japonica rice				
Gallic acid	Pericarp	tr	nd	tr
	Aleurone layer	3.2±0.3a# (72.9) ##	1.2±0.1 (27.1)	4.4±0.3a
	Embryo	1.3±0.1b (100)	nd	1.3±0.1b
	Endosperm	tr	nd	tr
Protocatechuic acid	Pericarp	9.0±0.5a (56.1)	7.1±0.8a (43.9)	16.1±1.4a
	Aleurone layer	10.5±0.8b (83.9)	2.0±0.2b (16.1)	12.5±0.5b
	Embryo	4.2±0.7c (100)	nd	4.2±0.7c
	Endosperm	tr	nd	tr
p-hydroxybenzoic acid	Pericarp	11.2±0.9b (39.4)	17.2±0.8b (60.6)	28.4±0.9b
	Aleurone layer	12.8±1.1a (38.6)	20.3±0.3a (61.4)	33.0±0.8a
	Embryo	12.2±0.8a (63.7)	7.0±0.1c (36.3)	19.2±0.8c
	Endosperm	2.0±0.1c (100)	nd	2.0±0.1d
Chlorogenic acid	Pericarp	nd	14.8±0.6b (100)	14.8±0.6c
	Aleurone layer	9.3±0.1 (36.2)	16.4±1.1b (63.8)	25.7±1a
	Embryo	nd	23.0±1.3a (100)	23.0±1.3b
	Endosperm	2.4±0.1 (20.7)	9.1±0.5c (79.3)	11.5±0.5d
Vanillic acid	Pericarp	1.9±0.6a (19)	8.2±0.6a (81)	10.1±1.1b
	Aleurone layer	4.0±1.7a (31.3)	8.8±1.6a (68.7)	12.8±0.2a
	Embryo	2.6±1.1a (100)	tr	2.6±1.1c
	Endosperm	tr	nd	tr
Caffeic acid	Pericarp	tr	tr	tr
	Aleurone layer	tr	nd	tr
	Embryo	9.7±1 (100)	nd	9.7±1
	Endosperm	nd	nd	nd
Syringic acid	Pericarp	11.9±0.7b (54.4)	10±0a (45.6)	22±0.7b
	Aleurone layer	13.1±1.6b (56.5)	10.1±0.3a (43.5)	23.2±1.9b
	Embryo	19.4±0.6a (67.3)	9.4±1a (32.7)	28.8±0.9a
	Endosperm	1.5±0.1c (100)	nd	1.5±0.1c
Coumaric acid	Pericarp	20.4±2.9b (2.1)	929.6±14.8a (97.9)	950±13.9a
	Aleurone layer	15.6±2.4c (1.8)	832.6±65.8b (98.2)	848.2±63.5b
	Embryo	35.7±2.9a (8.7)	373±10.2c (91.3)	408.7±7.8c
	Endosperm	1.5±0.2d (7.5)	18.9±0.4d (92.5)	20.5±0.2d
Ferulic acid	Pericarp	45.5±1.4b(2)	2192.7±38.3a (98)	2238.2±38.5b
	Aleurone layer	40.6±3.8b (1.5)	2692.2±166b (98.5)	2732.8±162.5a
	Embryo	140.4±6.7a (14.3)	843±12.3c (85.7)	983.4±9.3c
	Endosperm	4.7±0.1c (3.7)	123.9±4.2d (96.3)	128.6±4.2d
Isoferulic acid	Pericarp	1.2±0.3b(1.4)	86.2±6.6b(98.6)	87.4±6.8b
	Aleurone layer	tr	124.5±13.3a (100)	124.5±13.3a

	Embryo	4.8±2a (9.3)	46.4±7.4c (90.7)	51.1±8.9c
	Endosperm	nd	6.3±0.3d (100)	6.3±0.3d
Catechin	Pericarp	4.1±0.6b (100)	nd	4.1±0.6b
	Aleurone layer	6.2±1.3a (100)	nd	6.2±1.3a
	Embryo	tr	nd	tr
	Endosperm	nd	nd	nd
Epicatechin	Pericarp	5.7±3.1b (5)	109.2±4a (95)	114.9±7b
	Aleurone layer	tr	106.9±11.4a (100)	106.9±11.4b
	Embryo	612.5±34.2c (87.6)	86.8±3b (12.4)	699.4±36.1a
	Endosperm	tr	tr	tr
Quercetin	Pericarp	8.8±0.5c (28.1)	22.6±0.6a (71.9)	31.4±1.1a
	Aleurone layer	11.6±0.2b (37.1)	19.6±1.1b (62.9)	31.2±1a
	Embryo	15.4±1.8a (62.8)	9.1±0.3c(37.2)	24.5±2b
	Endosperm	2.6±0.4d (100)	tr	2.6±0.4c
Indica rice				
Gallic acid	Pericarp	1.2±0.1a (100)	nd	1.2±0.1a
	Aleurone layer	1.3±0.1a (100)	nd	1.3±0.1a
	Embryo	tr	nd	tr
	Endosperm	tr	nd	tr
Protocatechuic acid	Pericarp	1.6±0.2b (24.3)	5±0.7 (75.7)	6.7±0.7a
	Aleurone layer	1.8±0.2b (100)	nd	1.8±0.2b
	Embryo	7.3±0.8a (100)	nd	7.3±0.8a
	Endosperm	tr	nd	tr
p-hydroxybenzoic acid	Pericarp	5.1±0.1b (38.3)	8.3±0.3a (61.7)	13.4±0.3a
	Aleurone layer	6.3±0.1a (51.6)	5.9±0.4b (48.4)	12.1±0.5a
	Embryo	6.2±0.1a (100)	nd	6.2±0.1b
	Endosperm	1.6±0.1c (100)	nd	1.6±0.1c
Chlorogenic acid	Pericarp	nd	23.6±2.2b (100)	23.6±2.2b
	Aleurone layer	nd	17.9±2.5c (100)	17.9±2.5c
	Embryo	9.1±0.1 (23.5)	29.8±3.0a (76.5)	38.9±3.1a
	Endosperm	2.9±0.1 (26.7)	7.8±0.4d (73.3)	10.7±0.4d
Vanillic acid	Pericarp	tr	15.9±2.0a (100)	15.9±2.0a
	Aleurone layer	tr	3.3±2.2b (100)	3.3±2.2b
	Embryo	tr	tr	tr
	Endosperm	nd	nd	nd
Caffeic acid	Pericarp	1.7±0.6b (100)	nd	1.7±0.6b
	Aleurone layer	2.3±0.1ab (100)	nd	2.3±0.1b
	Embryo	3±0.2a (100)	nd	3±0.2a
	Endosperm	tr	nd	tr
Syringic acid	Pericarp	8±0.6a (34.8)	15±1.1a (65.2)	22.9±0.8a
	Aleurone layer	8±0.6a (43.8)	10.3±1.7b(56.2)	18.3±2.2b
	Embryo	5.9±0.2b (43.6)	7.6±0.7c (56.4)	13.5±0.9c
	Endosperm	1.2±0.1c (100)	nd	1.2±0.1d

Coumaric acid	Pericarp	10.2±2.0a (1.1)	929.2±97.6a (98.9)	939.3±98.0a
	Aleurone layer	6.3±0.5b (1.5)	408.1±117.5b (98.5)	414.4±117.4b
	Embryo	4.2±0.6c (2.9)	140.7±39.2c (97.1)	144.9±39.2c
	Endosperm	tr	11.5±0.8 (100)	11.5±0.8d
Ferulic acid	Pericarp	27.7±1.2b (1.2)	2216.7±229a (98.8)	2244.4±229.4a
	Aleurone layer	21.3±1.2c (1.6)	1304±370.3b (98.4)	1325.3±371.5b
	Embryo	36.8±1a (10.5)	313.3±84.4c (89.5)	350.1±85.3c
	Endosperm	3.5±0.3d (3.4)	101.3±3.8d (96.6)	104.8±4d
Isoferulic acid	Pericarp	tr	119.2±14.6a (100)	119.2±14.6a
	Aleurone layer	tr	81.7±25.8b (100)	81.7±25.8b
	Embryo	5.3±0.7 (21.2)	19.9±6.9c (78.8)	25.2±6.7c
	Endosperm	nd	6.9±0.9d (100)	6.9±0.9d
Catechin	Pericarp	5.1±1b (100)	nd	5.1±1b
	Aleurone layer	7.4±1.1a (100)	nd	7.4±1.1a
	Embryo	tr	nd	tr
	Endosperm	nd	nd	nd
Epicatechin	Pericarp	tr	150.3±16.6a (100)	150.3±16.6b
	Aleurone layer	tr	74.9±24b (100)	74.9±24.0c
	Embryo	459.2±17.2 (93.5)	32.2±10.2c (6.5)	491.3±25.5a
	Endosperm	tr	tr	tr
Quercetin	Pericarp	9.5±0.9b (26)	26.9±2a (74)	36.4±2.6a
	Aleurone layer	10.3±1.3b (39.9)	15.6±2.8b (60.1)	25.9±3.7b
	Embryo	16.3±1a (100)	nd	16.3±1c
	Endosperm	2.6±0.2c (100)	nd	2.6±0.2d

#Values with no letters in common in each column are significantly different ($p<0.05$)

Values in parentheses indicate percentage contribution to the total phenolic acids.

Table 3 ORAC antioxidant capacities (trolox equivalent / g dry weight) of four fractions in two types of whole brown rice.

Rice type	Tissue fraction	Free	Bound	Total
Japonica rice	Pericarp	297.1±32a# (49.4) ##	303.9±19.5a (50.6)	601.0±13.1a
	Aleurone layer	293.1±25.9a (54.1)	248.5±10.8a (45.9)	541.6±15.8b
	Embryo	110.3±1.2b (72.5)	41.9±4.7b (27.5)	152.2±5.7c
	Endosperm	18.7±0.4c (81.1)	4.3±0.5c (18.9)	23.0±0.8d
Indica rice	Pericarp	251.3±27.1a (50.8)	243±14.2a * (49.2)	494.3±15.6a *
	Aleurone layer	238.1±27.8a (54.7)	197.4±19.8a * (45.3)	435.5±8.4b *
	Embryo	99.5±25.6b (78.1)	28±7.2b* (21.9)	127.5±32.6c
	Endosperm	15±0.8c* (73.7)	5.3±0.3c * (26.3)	20.3±0.4d *

Values with no letters in common in each column are significantly different ($p<0.05$)

Values in parentheses indicate percentage contribution to the total ORAC value.

* Values of each same fraction of *Japonica* and *Indica* rice are significantly different ($p<0.05$)

Table 4 CAA antioxidant capacity (μmol QE/100 g DW) of four fractions in two types of whole brown rice.

Rice type	Tissue fraction	Free	Bound	Total
Japonica rice	Pericarp	147.8±9.3b# (28.6)##	368.6±19a (71.4)	516.4±28a
	Aleurone layer	93.4±8.5c (19.8)	379.4±23.9a (80.2)	472.8±39.7b
	Embryo	279.7±12.2a (83.1)	56.7±1.4b (16.9)	336.5±11.7c
	Endosperm	10.4±0.4d (44.3)	13.1±1.1c (55.7)	23.5±0.8d
Indica rice	Pericarp	117.1±14.3b* (29.4)	281.2±8.5b* (70.6)	398.3±13.3b*
	Aleurone layer	120.8±8b * (22.4)	419.1±17.7a (77.6)	539.9±27.4a*
	Embryo	359.5±10.6a* (70.5)	150.1±6.9c*(29.5)	509.5±13.7a*
	Endosperm	8±0.3c* (43.1)	10.6±0.5d* (56.9)	18.6±0.8c*

Values with no letters in common in each column are significantly different ($p<0.05$)

Values in parentheses indicate percentage contribution to the total CAA value.

* Values of each same fraction of *Japonica* and *Indica* rice are significantly different ($p<0.05$)

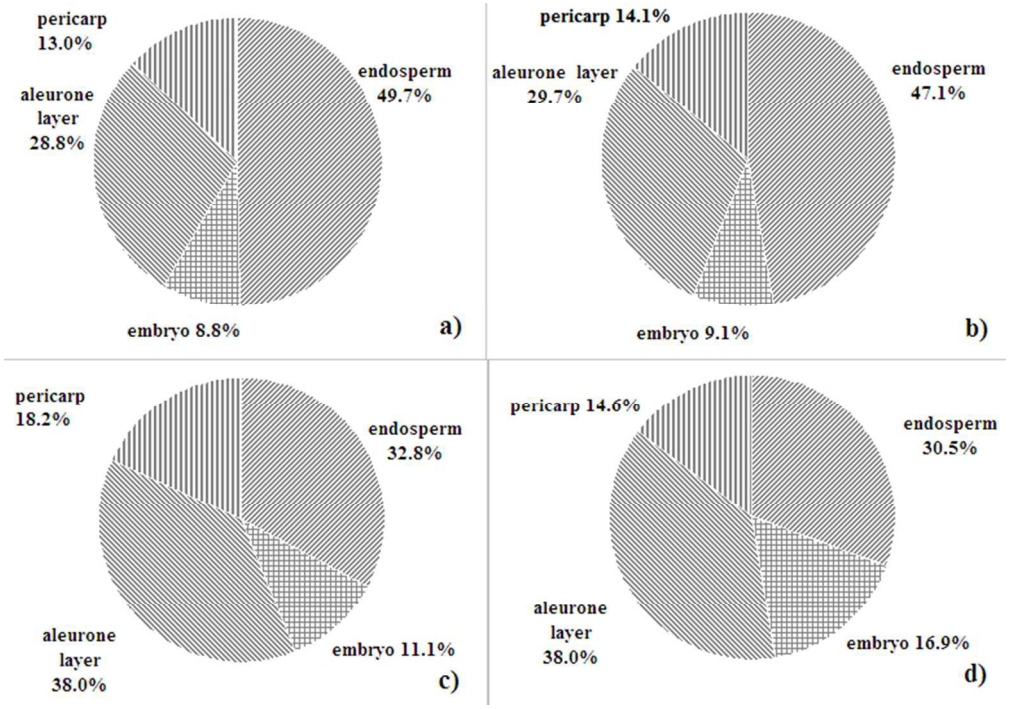
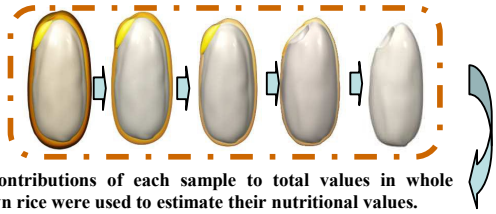


Figure 1: Percentage contributions of tissue fractions to total values (weight percentage*total values/sum of percentage contributions of embryo, aleurone layer and endosperm) **of (a) phenolics, (b) flavonoids, (c) ORAC, and (d) CAA in whole brown rice samples based on the naturally occurring proportions of pericarp, aleurone layer, embryo and endosperm fractions.**

Figure 2: Percentage contributions of each kind of sample to total values of weights, phenolics, flavonoids, ORAC and CAA in whole brown rice

*Samples	brown rice (a)	semi-brown rice (b)	embryo rice (c)	lightly milled rice (d)	polished rice (e)
**Weights	100%	98.0%	96.4%	95.7%	90.7%
**Phenolics	100%	87.0%	77.7%	64.1%	49.7%
**Flavonoids	100%	85.9%	76.0%	71.0%	47.1%
**ORAC	100%	81.8%	69.2%	51.8%	32.8%
**CAA	100%	85.4%	72.7%	49.5%	30.5%

*These samples are (a) brown rice, (b) semi-brown rice (detached pericarp/testa from brown rice), (c) embryo rice (detached pericarp/testa and a small percentage of aleurone layer from brown rice), (d) lightly milled rice (detached pericarp/testa, embryo and most of aleurone layer from brown rice), and (e) polished rice (detached pericarp/testa, embryo and most of aleurone layer from brown rice) commercially available based on the milling process. **Percentage contributions of whole brown rice, semi-brown rice (sum of percentage contributions of embryo, aleurone layer and endosperm), embryo rice (sum of percentage contributions of embryo, 2/3 aleurone layer and endosperm), lightly milled rice (sum of percentage contributions of 1/2 aleurone layer and endosperm) and polished rice (percentage contribution of endosperm) to total values of weights, phenolics, flavonoids, ORAC and CAA in whole brown rice were used to estimate their nutritional values.



Contributions of each sample to total values in whole brown rice were used to estimate their nutritional values.

Sample	Brown	Semi-brown	Embryo	Lightly	Polished
Weights	100%	98.0%	96.4%	95.7%	90.7%
Phenolics	100%	87.0%	77.7%	64.1%	49.7%
Flavonoids	100%	85.9%	76.0%	71.0%	47.1%
ORAC	100%	81.8%	69.2%	51.8%	32.8%
CAA	100%	85.4%	72.7%	49.5%	30.5%