



## Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins

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### ABSTRACT

Proanthocyanidin-containing rice varieties have been rarely reported. Antioxidant capacity, major antioxidant components, and nutritional parameters of eight traditional red-grained rice varieties containing proanthocyanidins grown in Sri Lanka were investigated. The tested traditional red varieties, on the average, had over sevenfold higher both total antioxidant capacity and phenolic content than three light brown-grained new-improved rice varieties. Major antioxidant phenolic compounds identified in this study included proanthocyanidins, phenolic acids and  $\gamma$ -oryzanols (ferulic acid derivatives). Proanthocyanidins were detected only in the traditional red varieties, but not found in new-improved ones. Most traditional red varieties also contained significantly higher levels of protein with well balanced amino acids and higher contents of fat, fibre and vitamin E (tocopherols and tocotrienols) than the new-improved ones. Great variations in antioxidant capacity, major phenolics, and nutritional parameters were observed among different rice varieties. These Sri Lankan traditional red-grained rice varieties containing proanthocyanidins may be used as important genetic sources for rice breeding.

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### 1. Introduction

Rice is the staple food of almost half the world population and increasingly becoming popular because of its nutritional and health beneficial properties. Rice cultivation is the main income source of millions of people in globe, particularly in Asia. Brown rice is unpolished whole grain that is produced by removing the husk and its colour may be light brown, reddish, purplish or black. Substantial amount of red rice are produced in south Asia including Sri Lanka. White (polished) rice is produced through stripping the bran layer (5%–8% of brown rice weight) of brown rice in the milling or whitening process. Bran layer is rich in protein, fibre, oil, minerals, vitamins, and phytochemicals and rice bran is a major by-product derived from rice milling (Yokoyama, 2004). In rice nutrition, rice protein has gained great attentions due to its relatively well-balance amino acid profile, which is superior in lysine content to wheat, corn, millet, and sorghum (Hegsted, 1969). High concentration of protein with well-balanced amino acid profile in rice has a great potential to improve human nutrition in rural families in Asia, which has become a major objective of rice breeding.

The health benefits of brown rice are attributed in part to their phytochemicals, mainly phenolic compounds, particularly in pigmented rice which has received the increasing attention because of its potent antioxidant properties. Phenolic compounds in dietary cereals possess potent antioxidant activity and provide health benefits associated with reduced risk of chronic diseases (Liu, 2004). The pigmented rice was reported to have higher phenolic content and stronger antioxidant activity than white rice (Shen, Jin, Xiao, Lu, & Bao, 2009; Zhang, Zhang, Zhang, & Liu, 2010). Major anthocyanin components of black rice were identified as cyanidin-3-glucoside and peonidin-3-glucoside and these compounds possessed notable antioxidant and anti-inflammatory activities (Hu, Zawistowski, Ling, & Kitts, 2003; Zhu, Cai, Bao, & Corke, 2010). In black rice, bran layer contained most of antioxidants including phenolic acids, anthocyanins,  $\gamma$ -oryzanols, vitamin E homologues, phytic acid, etc. (Kong & Lee, 2010; Zhang, 2000; Zhang et al., 2010). Moreover,  $\gamma$ -oryzanols, phytosteryl ferulates extracted from rice bran oil, has also been shown antioxidant properties which can improve the oxidative stability of different foods (Lerma-García, Herrero-Martínez, Simó-Alfonso, Mendonça, & Ramis-Ramos, 2009). Tan and Shahidi (2011) reported that phytosteryl ferulates possessed high antioxidant activity and may serve as cholesterol-lowering agents.

Proanthocyanidins naturally occur in some cereals and legume seeds (e.g., barley kernels, lentil) and are particularly abundant in

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many fruits (e.g., grape, apple, different berries) and they possess many beneficial healthy effects including superior antioxidant properties (Santos-Buelga & Scalbert, 2000), but have been rarely reported in grains of rice cultivars including conventional red-grained rice variety. Oki et al. (2002) found that major components in scavenging free radicals in red-hulled rice were procyanidins distributed in rice bran. Finocchiaro et al. (2007) reported that proanthocyanidins occurred in red rice which showed potent antioxidant capacity. Finocchiaro, Ferrari, and Gianinetti (2010) reported that among a set of Italian rice varieties, five red-grained varieties contained proanthocyanidins and no detectable anthocyanins, one black-grained variety contained anthocyanins and no detectable proanthocyanidins, but one black-grained variety had a large amount of both proanthocyanidins and anthocyanins. Additionally, Qiu, Liu, and Beta (2009) detected oligomeric procyanidins in commercial wild rice. Proanthocyanidins possess significantly stronger antioxidant activity than anthocyanins (Cai, Sun, Xing, Luo, & Corke, 2006).

Although cultivation and consumption of pigmented rice is limited in Western countries, traditional rice varieties with pigmented caryopsis are popularly planted and have long been consumed as healthy food in some Asian countries (e.g., Sri Lanka, India, China, Japan, etc.) (Ahuja, Ahuja, Chaudhary, & Thakrar, 2007; Finocchiaro et al., 2007; Finocchiaro et al., 2010). Since a long time ago, Sri Lankan people have believed that the traditional rice varieties (mainly red-grained rice) are superior in nutritional quality and healthy effect. The consumption of these traditional varieties may be helpful to reduce the occurrence of diet-related chronic diseases. However, nobody has carried out the comprehensive study on antioxidant activity and nutritional quality of the traditional red rice varieties in Sri Lanka. In our recent study on physicochemical and digestibility properties of brown and polished rice from Sri Lankan traditional and new-improved varieties (Gunaratne, Bentota, Cai, Collado, & Corke, 2011), it has unexpectedly been found that several traditional rice varieties have higher levels of phenolics and stronger antioxidant activity than new-improved rice varieties. Thus, it is necessary that more traditional rice varieties are collected for further study on their major antioxidant components and nutritional quality parameters.

This study aims to investigate and compare total phenolic content, antioxidant capacity, and major types of antioxidant phenolics and representative compounds (mainly proanthocyanidins and phenolic acids), as well as nutritional components (i.e., protein, amino acid profiles, vitamin E homologues, etc.) of eight traditional red-grained rice varieties and three light brown-grained new-improved rice varieties grown in Sri Lanka under the same climatic and agronomic practices. This study will provide valuable information to increase scientific understanding of Sri Lankan traditional red rice varieties as functional foods.

## 2. Materials and methods

### 2.1. Materials

Eight traditional red-grained rice varieties (Kalu Heenati, KAH; Sudu Heenati, SUH; Beheth Heenati, BEH; Rathu Heenati, RAH; Madathawal, MT; Kahatawee, KAW; Sulai, SU; and Molligoda, MG) and three light brown-grained new-improved varieties (BG250, BG300, and BG359) were obtained from the Rice Research and Development Institutes in Bombuwalla and Batalagoda, Sri Lanka, respectively. The moisture content of grains was brought to below 14% by drying in an air drier and stored at room temperature till use in experiments. Rice grains were dehusked using a dehusker (Satake Corporation, Hiroshima, Japan) and then polished using a polisher (Satake Corporation, Hiroshima, Japan). Bran was

removed by nearly 8% of total weight of grains and bran flour was collected separately. Then, brown and polished rice grains were milled to flour by a Cyclotec 1093 Sample Mill (Foss, Sweden). All flour samples were passed through a 0.5 mm sieve and stored in a sealed plastic box at room temperature till use.

2,2-Azinobis (3-ecthybenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulphate, sodium carbonate, and vanillin were purchased from Sigma/Aldrich (St. Louis, MO, USA), Folin-Ciocalteu reagent from BDH (Dorset, UK), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid) from Fluka Chemie AG (Buchs, Switzerland). Standards of ferulic acid, caffeic acid, *p*-coumaric acid, sinapinic acid, gallic acid, and catechin were from Sigma/Aldrich. The standard of  $\gamma$ -oryzanol ( $\geq 99\%$  purity) was purchased from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan) and the  $\gamma$ -oryzanol mixture with various components (mainly cycloartenyl ferulate, 2,4-methylene cycloartenyl ferulate, campesterol ferulate, and sitosterol ferulate) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Other chemicals and reagents used in this study were obtained from Sigma/Aldrich.

### 2.2. Determination of total antioxidant capacity and total phenolic content

Rice bran, and brown or polished rice flour sample (2 g) was extracted with 50 ml of 80% methanol at room temperature for 24 h in a water bath shaker (Shaking Bath 5B-16) (Techne, UK). The extract was filtered using a Millipore filter with a 0.45- $\mu$ m nylon membrane under vacuum at room temperature ( $\sim 23^\circ\text{C}$ ), and stored at  $4^\circ\text{C}$  until use. Total antioxidant capacity of the extract samples was assayed using the improved ABTS method (Shan, Cai, Sun, & Corke, 2005). Results were expressed in term of Trolox equivalent antioxidant capacity (TEAC), i.e., mmol Trolox/100 g dry weight (d.w.). Total phenolic content (TPC) of the extract samples was estimated using the Folin-Ciocalteu colorimetric method described by Shan et al. (2005). Results were expressed as mg gallic acid equivalents (GAE)/100 g d.w.

### 2.3. Determination of total proanthocyanidins

Each sample (0.5 g) of bran and brown rice flour was extracted in 4 ml of 80% methanol in a shaker (Shaking Heidolph Unimax 1010DT, Schwabach, Germany) at 300 rpm for 8 h, and then centrifuged at  $\sim 8000 \times g$  for 15 min. The supernatant was filtered through 0.2  $\mu$ m pore size syringe-filter, and then stored until use for identification and quantification of proanthocyanidins. Total proanthocyanidin content was measured using vanillin assay method (Sun, Ricardo-da-Silva, & Spranger, 1998) with some modification. The extract solution (0.4 ml) was mixed with 1 ml of sulfuric acid/methanol solution and 1 ml of 1% vanillin in methanol (w/v). A control mixture of the sample was prepared by adding 100% methanol instead of the vanillin solution for correcting the absorbance by nonvanillin reactive compounds to eliminate the influence of the interference (e.g., anthocyanins). After incubation for 15 min in a  $30^\circ\text{C}$  water bath, the absorbance of the sample and control mixtures was measured at 500 nm against a reagent blank and their difference was used to determine total proanthocyanidins of the samples, which was expressed as mg catechin (CE)/g sample (dry basis).

### 2.4. Determination of total phenolic acids

Extraction of phenolic acids (soluble and insoluble, bound and unbound, conjugated and unconjugated) for rice samples followed the alkaline extraction method reported by Liyana-Pathirana and Shahidi (2006), Liyana-Pathirana and Shahidi (2007) and Zhu

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