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Phenolic compounds and antioxidant properties of breeding lines between the white and black rice

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ABSTRACT

Advanced breeding lines made from the cross between the black and white rice as parents were collected to evaluate phenolic levels and antioxidant properties. No free phenolic acid was found in the soluble fraction, while *p*-coumaric acid, ferulic acid, isoferulic acid and vanillic acid were identified in insoluble bound fractions. Of noteworthy, is isoferulic acid which has rarely been reported to occur in cereal grains. Phenolic dehydrodimers were only observed in the insoluble bound fractions, which mainly consisted of 8-5′-coupled diferulic acids and 5-5′-coupled diferulic acids. Cyanidin 3-glucoside, peonidin 3-glucoside and cyanidin occurred in black and some light-purple rice samples. The breeding line YF53 has the highest total phenolic content (23.3 mg ferulic acid equiv./g), total anthocyanin content (2.07 mg cyanidin-3-glu equiv./g), and antioxidant activities. The results indicate that it is possible to develop advanced breeding lines for improvement of the phenolic profiles and antioxidant capacity with high yield.

1. Introduction

Rice (*Oryza sativa* L.) is the world's most important food crop and responsible for feeding approximately one-third of Earth's population. It is the dietary staple food in many Asian countries (Shen, Jin, Xiao, Lu, & Bao, 2009). Rice yields increased dramatically in China which contributes to about 31% of the world's rice production, due to the introduction of hybrid rice varieties (Li, Salas, DeAngelo, & Rose, 2006). Recently, many attempts have been made to develop better rice varieties, rich in certain functional compounds exhibiting antioxidant activities. Phenolics have been reported as the major hydrophilic antioxidants in rice while carotenoids, tocopherol, and gamma-oryzanols as the principle lipophilic antioxidative constituents (Min, McClung, & Chen, 2011). These substances have associated with the prevention of cardiovascular diseases, certain type of cancer and other diseases related to aging, thanks to their antioxidant properties (Kim, Tsao, Yang, & Cui, 2006; Shen et al., 2009). Thus, some type of rice varieties may be bred which has not only higher yield but also better quality containing increased levels of bioactive compounds.

Rice grain has a bark-like, protective hull, beneath which are the endosperm, bran and germ. Polishing of the dehulled rice to obtain milled rice, the form that is generally consumed, leads to loss of most of the nutritional components of the rice grain that are mostly deposited in the bran. Most rice varieties that are planted and consumed throughout the world have white pericarp. Therefore, more attentions should be paid to develop rice varieties with coloured pericarp or coloured rice bran layer (Nam et al., 2006; Qiu, Liu, & Beta, 2009). It has been shown that consumption of coloured rice causes decrease of oxidative stress and simultaneous increase of antioxidant capacity in the tested models (Hu, Zawistowski, Wenhua, & Kitts, 2003).

In China, attention has been paid to black rice that has an incredibly rich history known as "Forbidden Rice", because it was only reserved for the Emperor's consumption. Black rice has been characterised by the accumulation of phenolic acids, flavonoids and anthocyanins exhibiting antioxidant activities (Kaneda, Kubo, & Sakurai, 2006). However, black rice yield is lower than that of white rice which does not contain anthocyanins. Therefore, the novel rice varieties with high yield and good quality are expected to be bred through the hybrid between the white and black rice. Recently, 15 breeding lines with high yields have been bred through hybrid between the white (II32 B) and black (Yunanheixiannuo)

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rice as the parent. Their phenotypes in rice bran layer are easy to identify difference with colour shades, including black, light-purple and white. However, their functional properties and constitute of bioactive compounds need to be further studied.

The objective of this study was to investigate and compare phenolic compounds and antioxidant abilities among 15 offspring samples deriving from a cross between black and white rice. This study will be helpful for rice breeders to screen the hybrid samples with functional properties through comparative evaluation of phytochemicals profiles and antioxidant abilities among the earlier breeding line samples.

2. Materials and methods

2.1. Samples

A white rice (II32 B, YF43), a black rice (Yunanheixiannuo, a waxy rice, YF68) and 15 breeding lines derived from the cross between II32 B (YF43) and Yunanheixiannuo (YF68) were used in this study. The dehulled and unpolished grains of 15 breeding lines and the parent samples could be divided into three classes according to their colour shades: white (YF43, YF45, YF47, YF50, YF55, YF56), light-purple (YF44, YF49, YF62, YF63, YF67) and black (YF46, YF53, YF54, YF57, YF64, YF68) (Supplementary Fig. 1).

2.2. Chemicals

Folin–Ciocalteu reagent, DPPH, and ferulic acid were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). HPLC grade acetone and methanol were used in the extraction and fractionation. Phenolic acids standards (gallic acid, protocatechuic acid, *p*-hydroxylbenoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, isoferulic acid, *o*-coumaric acid), and anthocyanin and anthocyanidin standards (cyanidin chloride, delphinidin chloride, malvidin chloride, kuromanin chloride, callistephin chloride, idaein chloride, keracyanin chloride, cyanin chloride) were purchased from Sigma–Aldrich Chemical Co. Peonidin 3-*O*-glucoside chloride were purchased from Polyphenols Laboratories AS (Sandnes, Norway). MS grade acetonitrile, methanol and acetic acid were used in HPLC–MS/MS analysis. All of the HPLC grade and MS grade solvents were purchased from Sigma–Aldrich Chemical Co.

2.3. Extraction of free fractions

Rice flour (2 g) was extracted twice with 80% methanol at a ratio of 1:20 (w/v) for the soluble free phenolic compounds. Each time, the mixture was kept on a mechanical shaker (Thermo/Lab-Line/Barnstead MAX Q 4000, Artisian Scientific, Champaign, IL, USA) for 1 h at room temperature. After centrifuging (Model 2C5C, MANDEL, Guelph, ON) at 1430×*g* for 5 min, the supernatants obtained from each time were combined and concentrated to dryness by using a rotary evaporator (Bochi R-205, Flawil, Switzerland) at 35 °C. The dried methanol extract was redissolved in 5 mL of 50% methanol and the extracts were used as the soluble free fractions on analysis of phenolic compounds.

2.4. Extraction of insoluble bound fractions

The residue above obtained was washed with 40 mL of distilled water to eliminate organic solvent, and then filtered through a Whatman No. 1 filter paper. After drying in a hood at room temperature, the dried residue was hydrolysed with 40 mL of 4 M NaOH on a shaker (Thermo/Lab-Line/Barnstead MAX Q 4000, Artisian Scientific, Champaign, IL, USA) under nitrogen gas for 4 h. After digestion, the solution was adjusted to a pH 1.5–2.0 with 6 M HCl and then extracted with 70 mL of ethyl acetate for three times. The combined ethyl acetate fractions were evaporated to dryness and reconstituted in 5 mL of 50% methanol. The extracts were used as the insoluble bound fractions on analysis of phenolic compounds.

2.5. Extraction of anthocyanins

Extraction of anthocyanins was accomplished according to a modification of the methods reported in the literature (Hosseinian, Li, & Beta, 2008). Briefly, methanol acidified with HCl (1 N) (ratio 85:15, v/v) was added to rice flours (2 g) (sample to solvent ratio of 1:8) and the pH adjusted to 1.0. After shaking at 1800 rpm for 45 min, the supernatant was separated from the pellet by centrifuging at 5000*g*. The supernatant was evaporated to dryness at 40 °C and reconstituted in methanol (5 ml).

2.6. Colour determination

A Minolta spectrophotometer CM-3500d colorimeter (Minolta Co., Ltd., Osaka, Japan) with Spectra Magic version 3.6 software was used to measure the colour of rice samples. The colour was expressed using the L, a^*, and b^* colour space coordinates, where L represents lightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness.

2.7. Measurement of total phenolic content (TPC)

The TPC of crude extracts was evaluated by using modifications of the Folin–Ciocalteu method (Singleton & Rossi, 1965). Briefly, 200 μL of the appropriate dilutions of crude extracts was reacted with 1.8 mL of 10-fold diluted Folin–Ciocalteu reagent, which was freshly made. The mixture was then neutralised with 1.8 mL of sodium carbonate (60 g/L). The absorbance was measured at 725 nm after 90 min of reaction at room temperature. Ferulic acid was used as the standard. Results were expressed as mg of ferulic acid equivalents (FAE) per gram of rice (dry weight basis).

2.8. Determination of total anthocyanin content (TAC)

The total anthocyanins content was determined according to a pH-differential method previously described by Al-Farsi, Alasalvar, Morris, Baron, and Shahidi (2005) and modified by Li and Beta (2011). Briefly, 1 mL of anthocyanin extract was respectively diluted with pH 1.0 buffer and pH 4.5 buffer to a 25-mL final volume. The absorption of the diluted sample was measured at 510 nm and 700 nm. The results were expressed as mg of cyanidin 3-glucoside (cy 3-glu) equivalents per gram of rice (dry weight basis).

2.9. Determination of DPPH radical scavenging activity

This assay was based on the method of Brand-Williams, Cuvelier, and Berset (1995) as modified by Li, Pickard, and Beta (2007). Briefly, 200 μL of crude extract (or fraction) was added to 3.8 mL of 60 μM DPPH radical solution, which was freshly made. After 60 min of incubation at room temperature, the absorbance at 515 nm was measured. DPPH free radical scavenging activities of crude extracts were expressed as μM of trolox equivalents (TE) per gram of rice (dry weight basis) using a standard curve of trolox.

2.10. Evaluation of oxygen radical absorbance capacity (ORAC)

The ORAC assay was based on the method described by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) and modified by Li et al. (2007). A Precision 2000 automated microplate pipetting