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Effect of extrusion on phytochemical profiles in milled fractions of black rice

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ABSTRACT

The phytochemical profile and antioxidant activities of unprocessed and extruded milled fractions of black rice were investigated. Extrusion increased the free phenolics, anthocyanins and oxygen radical absorbance capacity (ORAC) and decreased the bound forms. The total phenolics, anthocyanins and ORAC increased by 12.6%, 5.4% and 19.7%, respectively, in bran. Extrusion decreased both free and bound phenolics and anthocyanins while ORAC values decreased by 46.5%, 88.4% and 33.1%, respectively, in polished rice and by 71.2%, 87.9% and 14.7%, respectively, in brown rice. A total of seven phenolics, gallic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric and ferulic acids, were detected in both forms. Cyanidin 3-glucoside (Cy-3-G), cyanidin 3-rutinoside and peonidin 3-glucoside were also detected with Cy-3-G found in the highest amounts in unprocessed and extruded rice bran. These results provide the basis for the development of different milled fractions of extruded black rice with balanced nutritional characteristics for today's functional food markets.

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1. Introduction

Epidemiological studies have consistently shown that the regular consumption of foods high in whole grains may contribute to maintaining good health and possibly reduce the risk of chronic diseases such as cardiovascular disease, type II diabetes, obesity and cancer (Okarter & Liu, 2010). Whole grain phytochemicals are assumed to be the key source of the health benefits of whole grains (Wang, Chen, Xie, Ju, & Liu, 2013).

Whole-grain phytochemicals consist of carotenoids (lutein, zeaxanthin, cryptoxanthin, and carotene), phenolics and vitamin E. Phenolics include phenolic acids (*p*-coumaric, caffeic, ferulic, vanillic, and syringic acids) as well as flavonoids (flavonols, flavonones, catechins, and anthocyanins) (Goufo & Trindade, 2014; Liu, 2007). Whole-grain phenolics possess potent antioxidant activities and are able to scavenge free radicals that may increase oxidative stress and potentially damage large biological molecules such as lipids, proteins and DNA. Black rice is a kind of typical whole grain, and is considered a good source of fiber, minerals and phytochemicals as well as basic nutrients (Zhang, Zhang, Zhang, & Liu, 2010). Traditionally, black foods, particularly black rice, have long been favored by Chinese people. The popularity of black rice is partly due to its particular phytochemicals, especially anthocyanins, which have been shown to have beneficial effects in preventing chronic diseases. As consumers' awareness of health increases, new processed foods containing black rice materials (rice bran, polished rice and brown rice of black rice) require the presence of bioactive ingredients to satisfy the demands of health-conscious consumers.

The extrusion of whole grains is becoming more popular in the food and pharmaceutical industries. Extrusion is a technology that affects the microstructure, chemical characteristics, and macroscopic shape of a product. It is a mechanical process that exposes the extruded material to high temperatures, shear forces and pressures over a short period of time. The temperatures encountered by the food material in the barrel of the extruder are high enough to gelatinize starch, denature protein and form complexes between starch, lipids and proteins (Wolfe & Liu, 2003). In particular, phenolics may undergo various changes, thus altering the antioxidant activity of the products. Many studies have reviewed and documented the effects of extrusion on the phenolic contents and antioxidant activity of different foods included thermally processed legumes (Han & Baik, 2008; Xu & Chang, 2008) and extruded barely (Sharma, Gujral, & Singh, 2012). It is also well documented that the total phenolic content and antioxidant activity of table beets, green beans and corn were affected by thermal processing (Jiratanan & Liu, 2004; Parra, Saldivar, & Liu, 2007).

Beneficial phytochemicals in grain and processed grain samples are distributed in rice in the free, soluble-conjugated and bound

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forms. Most of these free and bound compounds are found in different parts of the grain, particularly in distinct fractions obtained from milling the grains (Onyeneho & Hettiarachehy, 1992). A previous study investigated the phenolics and anthocyanin phytochemicals and their antioxidant activity in different milled fractions (bran, polished rice and brown rice) of black rice (Kong & Lee, 2010). It should be noted that the extraction procedure used in this study may have led to an underestimation of the total phenolics and antioxidant activity because the bound fraction was not included in the analysis (Butsat & Siriamornpun, 2010). Thus, a better understanding of how thermal processing influences the stability of phenolics in black rice and their distribution in the extrudate is required.

The objectives of the present investigation were to determine the content of free and bound phytochemicals (phenolics and anthocyanins) and their antioxidant activities and to analyze the composition and content of individual phytochemicals in both free and bound forms in different milled fractions (bran, polished rice and brown rice) of black rice before and after extrusion.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade methanol (MeOH), ethanol (EtOH), hexanes, ethyl acetate, hydrochloric acid (HCl), acetic acid (HAc), potassium chloride (KCl), sodium acetate (NaAc), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), fluorescein disodium salt, apigenin, sodium borohydride ($NaBH_4$, reagent grade), chloranil (analytical grade), vanillin (analytical grade), and catechin hydrate were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Tetrahydrofuran (THF, analytical grade) and aluminum chloride ($AlCl_3 \cdot 6H_2O$, analytical grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), trifluoroacetic acid (TFA, chromatographic grade) and acetonitrile (chromatographic grade) were purchased from Sigma. Gallic acid was purchased from ICN Biomedicals Inc. (Aurora, OH, USA). 2,2′-Azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals USA Inc. (Richmond, VA, USA). Cyanidin 3-glucoside (Cy-3-G), cyanidin 3-rutinoside (Cy-3-R), and peonidin 3-glucoside (Pe-3-G) were purchased from Polyphenols Laboratories (Sandens, Norway).

2.2. Grain samples and sample preparation

Black rice samples (*Oryza sativa* var. Heiyounian) were obtained from the Experimental Station of the Rice Research Institute of Guangdong Academy of Agricultural Sciences in 2012. They were sown in late March and harvested in mid-July. The rice grains were then air-dried to a moisture content of approximately 13% and stored at room temperature for 3 months. The rice samples were milled to separate the husk from the brown rice. The husk was not included in the analysis. The brown rice was then polished using a rice milling machine (Satake Co., Hiroshima, Japan) to obtain approximately 10% (w/w) rice bran (bran/embryo) and approximately 90% (w/w) polished rice. The bran, polished rice and brown rice samples were sieved by passing through a 60-mesh sieve using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO, USA) and stored at −40 °C until further analysis.

2.3. Extrusion

Extrusion was performed using a Werner and Pfleiderer Continua 37 co-rotating twin-screw extruder (Stuttgart, Germany). The screw diameter, (L/D) ratio and die diameter were 37 mm, 27/1 and 6 mm, respectively. The feed rate (25 kg/h) and screw speed (200 rpm) were kept constant. The extrusion was carried out at 120 °C with the temperature of different barrel zones set at 60, 100 and 120 °C. The feed moisture was conditioned to 12–17%. The extrudates were cooled to room temperature, packed in polyethylene bags and later milled to flour using a grinder (Sujata, India) to a particle size <250 μm and stored at −20 °C until further analysis.

2.4. Extraction of free phenolic compounds

Free phenolic compounds in the raw or extruded flours were extracted using the method of Sun, Chu, Wu, and Liu (2002). For each sample, a total of 0.5 g rice bran and 2 g polished/brown rice were blended with 50 mL of chilled acidified methanol (95% methanol and 1 M HCl 85:15, v/v). The extract was homogenized using an XHF-D homogenizer at 10,000 rpm for 5 min in an ice bath (Ningbo Xin-zhi-Bio Technology Co. Ltd., Ningbo, China) and centrifuged at 2500×*g* for an additional 10 min. The extraction was repeated. The supernatants were removed, pooled and concentrated by evaporation at 45 °C. The free phenolics were then made up to a final volume of 10 mL with chilled acidified methanol and stored at −20 °C until further analysis.

2.5. Extraction of bound phenolic compounds

The bound phenolic compounds in the different milled fractions of black rice and their extrudates were extracted according to the methods of Naczk and Shahidi (1989) and Sun et al. (2002). The residue from the free extraction was then digested with 40 mL of 2 M NaOH at room temperature. The oxygen was removed under nitrogen gas and the sample was shaken for 1 h. The mixture was extracted using hexanes to remove lipids and then neutralized with concentrated hydrochloric acid. The remaining extract was then extracted 5 times with ethyl acetate. The combined supernatants were evaporated under vacuum at 45 °C to dryness. The bound phenolics were reconstituted with distilled water to a final volume of 10 mL and then stored at −20 °C until further analysis.

2.6. Determination of total phenolic content

The total phenolic content of each extract was determined using the Folin–Ciocalteu (FC) colorimetric method described by Dewanto, Wu, Adom, and Liu (2002). Briefly, 0.5 mL of distilled water and 0.125 mL of the above extract were added to a test tube. FC reagent (0.125 mL) was added to the mixture and it was allowed to react for 6 min. Then, 1.25 mL of 7% aqueous sodium carbonate solution and distilled water were added to make up the total volume to 3 mL. The mixture was incubated for 90 min at room temperature. The absorbance was read at 760 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). Free, bound and total phenolic contents were measured by comparison to a standard curve of gallic acid solutions and were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (DW) of sample.

2.7. Determination of total anthocyanin content

The total anthocyanin content of each extract was determined using a spectrophotometric pH differential protocol adapted from that of Wolfe and Liu (2003). Briefly, the above extracts were mixed thoroughly with 0.025 M potassium chloride (pH 1) buffer.

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