Food Research International 76 (2015) 813–820

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effects of cooking and in vitro digestion of rice on phenolic profiles and antioxidant activity

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 17 July 2015
Accepted 19 July 2015
Available online 29 July 2015

Keywords:
Polished rice
Brown rice
Phenolics
Flavonoids
Phenolic acids
Antioxidant activity

ABSTRACT

The aim of this study was to analyze the content and distribution of free and bound phenolic components and the oxygen radical absorbance capacity (ORAC) antioxidant activity of brown and polished rice after cooking and in vitro digestion. The results showed that cooking decreased the free and bound phenolic and flavonoid contents and ORAC values. Cooking decreased the total phenolic content of brown and polished rice by 47.9% and 71.0%, total flavonoid content by 72.8% and 82.5%, and total ORAC values by 31.8% and 72.9%, respectively. After in vitro digestion, the total phenolic content, total flavonoid content, and total ORAC values had increased by 195.6%, 34.6% and 185.7%, respectively, in brown rice, and by 403.3%, 13.1% and 293.4% respectively, in polished rice, compared with the cooked samples. Seven individual phenolics (gallic, protocatechuic, chlorogenic, caffeic, syringic, coumaric, and ferulic acids) were detected in both free and bound forms. The results showed that the phytochemical content and its antioxidant activity after cooking will be underestimated if the bound fraction after in vitro digestion is not included in calculations. The results of this study show that cooking affects the phenolic content and antioxidant capacity of rice. However, the in vitro digestion process could actually improve the digestion and absorption of beneficial components of cooked rice at the intestinal level.

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1. Introduction

The consumption of whole cereal grains is strongly recommended because of their protective effects against many chronic diseases such as cancer and cardiovascular diseases. Cereal grains provide a rich mixture of phytochemicals, and regular intake of these grains has health benefits in humans. Until recently, there has been less attention paid to whole grain consumption than to fruit and vegetable consumption. Phytochemicals in cereal grains consist of carotenoids (lutein, zeaxanthin, cryptoxanthin and carotene), and phenolics (phenolic acids, flavonols, flavonones and catechins) (Wang, Chen, Xie, Ju, & Liu, 2013). As one of the largest groups of phytochemicals in cereal grains, phenolics are distributed in both free and bound forms. The bound form is covalently conjugated to the cell wall materials through ester bonds and can survive in the gastrointestinal tract, finally being released by bacterial fermentation in the colon to exert a localized health benefit (Das & Singh, 2015; Naczk & Shahidi, 1989; Vitaglione, Napolitano & Fogliano, 2008).

Consumers' awareness of the health benefits of whole cereal grains has increased in recent years. This has resulted in an increasing demand for convenient, ready-to-use whole cereal grain products. However, processing methods can greatly affect the quantity and quality of phytochemicals. One study reported that thermally processed corn had lower anthocyanins and phenolic compound content, although the thermal process may have helped to release bound phenolic acids (Dewanto, Wu, Adom, & Liu, 2002; Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014). In other studies, thermal processing significantly decreased the total phenolic content, anthocyanin content, and antioxidant activity in black rice (Hiemori, Koh, & Mitchell, 2009), and changed the free and bound phenolic content and antioxidant capacities in brown, purple, and red rice bran (Min, McClung, & Chen, 2014). However, the effects of thermal processing on polished rice have not been reported. Different extraction systems also affect the detection of these phytochemicals and their antioxidant activity (Adom, Sorrells, & Liu, 2003; Yu et al., 2002; Zielinski & Kozlowska, 2000). Thus, it is important to determine how thermal treatment of brown rice and polished rice affects their free and bound phenolic profiles and their antioxidant activity.

Previous studies on the antioxidant activity of rice have not incorporated an in vitro digestion model. Because digestion can affect the functional properties of bioactive compounds, it is important to evaluate the free and bound phenolic compounds in foods after digestion. Recent research has elucidated the functional properties of phenolics, flavonoids, anthocyanins and antioxidant activity after in vitro gastrointestinal digestion of vegetables, fruits, and their extracts (Bouayed, Hoffmann, & Bohn, 2011; Faller, Fialho, & Liu, 2012; Sancho & Pastore, 2012). From

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http://dx.doi.org/10.1016/j.foodres.2015.07.032
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the literature, we can conclude that in vitro digestion affects the total phytochemical content and their antioxidant activity. For example, the phenolics and flavonoids in *feijoada* (beef and pork stewed with beans) were unaffected by digestion, but the antioxidant activity of *feijoada* was higher before digestion than afterwards (Faller et al., 2012). In vitro digestion also increased the antiradical activity of wheat bread samples (Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009). In the present study, an in vitro model of the gastrointestinal tract was used to simulate the digestion process to assess any changes in the antioxidant activity of extracts from brown and polished rice.

Therefore, the objectives of the present study were as follows: (1) to investigate changes in the free and bound phytochemical (phenolics and flavonoids) contents of rice samples and their antioxidant activity; and (2) to characterize changes in the composition and content of individual phenolics in free and bound forms in brown and polished rice after cooking and in vitro digestion. This information on the changes in antioxidant components of rice after in vitro digestion and cooking is important for evaluation of human nutrition.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade methanol (MeOH), hexane, ethyl acetate, hydrochloric acid (HCl), 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). Apigenin, 2′, 7′-dichlorofluorescin diacetate (DCFH-DA), fluorescein disodium salt, sodium borohydride ($NaBH_4$, reagent grade), chloranil (analytical grade), vanillin (analytical grade), and catechin hydrate were purchased from Sigma-Aldrich (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 (FC), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), trifluoroacetic acid (TFA, chromatographic grade), and acetonitrile (chromatographic grade) were also purchased from Sigma. Gallic acid was purchased from ICN Biomedicals (Aurora, OH, USA), and 2, 2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals (Richmond, VA, USA). Pepsin (from porcine stomach mucosa), pancreatin (from porcine pancreas), and bile extract (from porcine) were purchased from Sigma-Aldrich.

2.2. Grain samples and sample preparation

The rice variety tested was TianYou 998, which was obtained from the experimental fields of Guangdong Academy of Agricultural Sciences in Guangzhou. Rice seeds were sown in late March 2013. The plants were harvested in mid-July, and then threshed. The rice grains were dried in the sun and stored at room temperature. The rice samples were milled to separate the husk from the brown rice. To produce polished rice, the brown rice was polished using a rice milling machine (Satake Co., Hiroshima, Japan). The samples of brown and polished rice were packed in plastic bags and stored at −20 °C. Each sample was analyzed in parallel using three replicates.

2.3. Cooking process

Cooking conditions were optimized by preliminary experiments carried out for this treatment, which made rice reaching tenderness for adequate palatability and taste was used. Brown and polished rice samples were placed in an electrically heated pressure cooker (rice/water, 1:2, w/v) and cooked for 20 min. The cooked rice was thoroughly freeze-dried and stored at −20 °C until analysis. Three replicates were performed for the cooking process.

2.4. Gastrointestinal in vitro digestion

This method was adapted from Faller et al. (2012) and Gawlik-Dziki et al. (2009) with a few modifications. Briefly, 20 g cooked rice was blended with 55 mL saline solution (140 mM NaCl, 5 mM KCl, and 150 μM BHT). The mixture was homogenized at 10,000 r/min for 5 min using an XHF-D homogenizer (Ningbo Xin-Zhi-Bio Technology Co., Ningbo, China) at room temperature, and then acidified to pH 2.0 using 0.1 M/1 M HCl. Then, the sample was mixed with 2 mL pepsin solution (0.2 g pepsin dissolved in 5 mL 0.1-mol/L HCl) and incubated in a thermostatic water bath at 37 °C for 1 h. For intestinal digestion, the pH of the digestate was adjusted to 7.0 using 1M$NaHCO_3$. Then, 20 mL pancreatin bile solution (0.9 g of bile extract and 0.15 g of pancreatin in 75 mL of 0.1-M $NaHCO_3$) was added, and the mixture was incubated in a thermostatic waterbath at 37 °C for 2 h. The digested solution was freeze-dried to obtain the in vitro-digested sample of cooked rice. Digestion with simulated gastric and intestinal fluids was performed in triplicate.

2.5. Extraction of phenolics

2.5.1. Extraction of free phenolics

The method was adapted from Sun, Chu, Wu, and Liu (2002). Each raw, cooked, or digested rice sample (2 g) was blended with 50 mL acidic methanol solution (95% methanol: 1 mol/L HCl = 85:15, v/v, precooled at 5 °C). The extract was homogenized using the XHF-D homogenizer at 10,000 rpm for 5 min in an ice bath and centrifuged at 2500 × g for a further 10 min. This extraction procedure was repeated, and the supernatants obtained from the two centrifugations were pooled and evaporated at 45 °C. The resulting residue was dissolved in 10-mL methanol to give the free phenolic extract solution, which was stored at −20 °C until analysis. All analyses were performed in triplicate.

2.5.2. Extraction of bound phenolics

The method was adapted from Adom et al. (2003) and Naczk and Shahidi (1989). The residue from the free extraction was hydrolyzed using 40-mL 2 mol/L NaOH solution. The solution obtained was protected by nitrogen and shaken at room temperature for 1 h. The solution was then neutralized with concentrated hydrochloric acid, degreased with 100 mL *n*-hexane, and then extracted five times with 100 mL ethyl acetate. All extract solutions were pooled and evaporated at 45 °C. The resulting residue was dissolved in 10 mL methanol to give the bound phenolic extract solution, which was stored at −20 °C until analysis. All analyses were performed in triplicate.

2.5.3. Determination of total phenolic content

The method was adapted from Singleton, Orthofer, and Lamuela-Raventos (1999). A 0.125-mL aliquot of the free or bound phenolic extract solution was reacted with 0.5 mL distilled water and 0.125 mL FC reagent for 6 min. Then, 1.25 mL 7% (m/v) Na_2CO_3 solution was added to the mixture to complete the volume to 3 mL. The resulting solution was incubated for 90 min and then its absorbance was measured at 760 nm using a UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). Gallic acid was used as the standard and the total phenolic content was expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW). All analyses were performed in triplicate.

2.6. Determination of total flavonoid content

The total flavonoid content was determined as described by Jia, Cheng, and Wu (1999), Min, Gu, McClung, Bergman, and Chen (2012), and Vichapong, Sookserm, Srijesdaruk, Swatsitang, and Srijaranai (2010) with modifications. Free or bound phenolic extract solution (0.3 mL) was reacted with 1.5 mL distilled water and 0.09 mL 5% (m/v) $NaNO_2$ solution for 6 min at 25 °C. Then 0.18 mL 10% (m/v) $AlCl_3 \cdot 6H_2O$ was added. After 5 min, 0.6 mL 1-mol/L NaOH solution was added to

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