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Changes of phenolic-acids and vitamin E profiles on germinated rough rice (*Oryza sativa* L.) treated by high hydrostatic pressure

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ABSTRACT

This study was performed to investigate changes in the phenolic acid and vitamin E profiles of germinated rough rice following high hydrostatic pressure treatment (HPT). Rough rice was germinated at 37 °C for two days and subjected to 0.1, 10, 30, 50, and 100 MPa pressures for 24 h. The total phenolic acid content increased from 85.37 μg/g at 0.1 MPa to 183.52 μg/g at 100 MPa. The highest gallic acid (4.29 μg/g), catechin (9.55 μg/g), *p*-coumaric acid (8.36 μg/g), ferulic acid (14.99 μg/g), salicylic acid (14.88 μg/g), naringin (6.18 μg/g), *trans*-cinnamic acid (45.23 μg/g), and kaempferol (40.95 μg/g) contents occurred in the sample treated at 100 MPa after germination. The maximum vitamin E content of about 2.56 (BG) and 4.34 mg/100 g (AG) were achieved at 30 MPa. These result suggest that a combination of HPT and germination are efficient method for enhancement of functionality in rough rice, and clarify the influence of HPT conditions on the vitamin E and phenolic acid in germination rough rice.

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1. Introduction

One of the most produced and consumed grains in the world, i.e., rough rice (*Oryza sativa*), is a rich source of bioactive compounds including many antioxidants, such as phenolic acid, vitamin E, and γ-oryzanol (Mira, Massaretto, Pascual, & Marquez, 2009).

Antioxidants in whole cereal grains are considered to be major contributors to the health benefits of these foods. Whole grain (i.e., unmilled, brown, rough) rice is a good source of antioxidants, most of which are present in the bran and hull fractions. Lipophilic antioxidants, which include vitamin E homologues (including α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols) and γ-oryzanol, are abundant in whole grain rice, and their health benefits have been well-documented (Jariwalla, 2001; Rui, 2007). Phenolic compounds, such as phenolic acids and flavonoids, are also abundant in whole grain rice (Goffman & Bergman, 2004). Phenolics have been investigated extensively because they exhibit a diverse range of bioactivities, such as antioxidant (Arab, Alemzadeh, & Maghsoudi, 2011), antimicrobial (Kondo, Teongtip, Srichana, & Itharat, 2011), antiviral (Ray et al., 2013), and anti-inflammatory properties (Akihisa et al., 2000), and promote overall human health.

Germination improves the quality of cereal for digestibility and physiological functions (Yang, Basu, & Ooraikul, 2001). Germination activates the dormant enzymes in rice grain and induces the hydrolysis of high–molecular weight polymers resulting in the generation of antioxidant and bioactive compounds such as, vitamin E, phenolic compounds, GABA, γ-oryzanol, and useful amino acids (Yao, Yang, Zhao, & Xiong, 2008). Kim, Hwang, Kim, Woo, and Jeong (2012) reported changes in the chemical and functional components, such as free sugars, free fatty acids, phytic acid, vitamin E, γ-oryzanol, and GABA, in rough rice before and after germination. In addition, changes in the antioxidant (Kim et al., 2011a), anticancer (Kim et al., 2010), and enzyme inhibitory activities (Kim et al., 2013a, 2013b) of rough rice with differing germination periods have been reported.

Initially exploited in chemistry and physics, high hydrostatic pressure treatment (HPT) has gained importance in various biology-related fields. In particular, it has been investigated as an alternative to high-temperature treatment in food and pharmaceutical industries and as a tool to study the relationships between structure and function in biochemical systems (Mozhaev, Heremans, Frank, Mansson, & Balny, 1996). HPT can cause molecular and structural changes, such as cell deformation, cell

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membrane damage, stabilization of enzymes, and protein denaturation, in structurally fragile materials (Mozhaev et al., 1996). HPT has been reported as an effective method for extracting bioactive compounds, such as anthocyanins (Corrales, García, Butz, & Tauscher, 2009) and caffeine (Jun, 2009), from natural resources.

The main parameters that affect HPT are classified as either process parameters (such as pressure, temperature, time, etc.) or environmental factors (i.e., moisture content, pH, growth conditions of microorganisms, and enzyme production, for example). Among these, the pressure applied is the most important variable for increasing the efficiency of extraction of functional components from plant cells (Koseki & Yamamoto, 2006).

Antioxidants, including vitamin E homologues and phenolics, are abundant in whole grains. However, the majority of antioxidants in rough rice are present as insoluble, cell wall–bound forms which have not been considered in most antioxidant-related studies regarding rice. In previous study, HPT in combination with germination was suggested as an efficient method to significantly modify the extractability of functional compounds and accelerate the biosynthesis of physiological metabolic material because HPT enhances the enzymatic reaction rate.

In the present study, we report investigation into the application of HPT to enhance the antioxidant properties of rough rice. Also, the influences of various pressure strengths (0–100 MPa) on changes in antioxidant content of germinated rough rice were investigated.

2. Materials and methods

2.1. Germinated rough rice preparation

Rough rice (cv. Ilpumbyeo, *O. sativa* L.) was grown at Jeungpyeong, Chungbuk, Korea during the 2011 growing season. For germination, the seed was soaked in distilled water (seed water ratio, 1:5, w/v) at 20 °C for three days; the water was changed every 24 h. The soaked seeds were placed in trays and germinated in a seed germinator (WGC 450, Dahan Inc., Seoul, Korea) for two days at 37 °C and 80% relative humidity (Kim et al., 2011a, 2011b).

2.2. High hydrostatic pressure treatment

HPT of the germinated rough rice was carried out using a pressure treatment system (super-high-pressure liquefying system, TFS-2L, Inoway, Inc., Anyang, Korea) compressed using fluid; the temperature of the pressure chamber was maintained at 37 °C under the same conditions as germination. The germinated rough rice (20 g) was mixed with 20 mL of distilled water. The mixtures were transferred to an aluminum foil-laminated film (Newpack, Seoul, Korea) and heat-sealed in vacuum packaging (Chamber-type vacuum package, DP-901, Dew Pack Korea machinery Co., Seoul, Korea.). The packaged samples were subjected to 0.1, 10, 30, 50, and 100 MPa of pressure at 37 °C for 24 h. HPT was carried out immediately after germination to prevent enzyme inactivation. All samples were dried at 50 °C for two days using a hot air dryer (WFO-459PD, EYELA, Tokyo, Japan) and stored at −20 °C in a deep freezer (ultralow temperature freezer, MDF-393, Sanyo, Akaiwa, Japan). Also, the samples were ground using the hammer mill (100 mesh) before analysis of phenolic acid and vitamin E.

2.3. Determination of phenolic acids

The phenolic acid contents were measured according to the methods described by Seo, Ko, and Song (2011) and Jung, Hong, and Cho (2012) with slight modifications. The powdered samples (4 g) were extracted three times with 90% methanol (40 mL) at 25 °C for 1 h using an ultrasonic bath (SD-350H; Seong Dong, Seoul, Korea). The extracts were then filtered using the watman No.4 filter paper, combined, and concentrated using a rotary evaporator under vacuum. The residue was dissolved in 5 mL of distilled water and extracted three times with 10 mL of diethyl ether/ethyl acetate solvent (50:50, v/v) using the phase separation between water and ether/ethyl acetate solvent. The supernatant layer was evaporated using a rotary evaporator under vacuum. The residues were dissolved in methanol and filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA). The phenolic acid compositions were determined using an HPLC system. The analytical column was an ODS column (5 μm, 4.6 mm × 250 mm, Agilent Technologies). Gradient elution was employed using solvent A (water containing 0.1% (v/v) acetic acid) and solvent B (acetonitrile containing 0.1% (v/v) acetic acid). The gradient program was as follows: 0–2 min, 92% to 90% A in B (gradient); 2–27 min, 90% to 70% A in B (gradient); 27–50 min, 70% to 10% A in B (gradient); 50–51 min, 10% to 0% A in B (gradient); 51–60 min, 0% A in B (isocratic); and 60–70 min, 0–92% A in B (gradient). The flow rate was kept at 1 mL/min, and the injection volume was 20 μL. The UV detector was set at 280 nm. The phenolic acid standard mixture containing gallic acid, chlorogenic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, rutin, ferulic acid, salicylic acid, naringin, hesperidin, myricetin, quercetin, *trans*-cinnamic acid, naringenin, and kaempferol was prepared in HPLC-grade methanol. The concentrations of phenolic acid were determined by standard curves obtained by injecting different concentrations (10–100 μg/mL) of the phenolic acid standard. Peaks were verified by adding the standard phenolic acids to the samples, and each peak area was calculated in relation to a standard peak area. The total phenolic acid content was calculated by adding the amounts of all phenolic acid components.

2.4. Determination of vitamin E

The vitamin E contents in the samples were determined using the direct solvent extraction method (Lee, Landen, Phillips, & Eitenmiller, 1998). A 2.5 g sample was added to a 50 mL conical tube and mixed with 2 mL of hot water (80 °C) after which 5 mL of isopropanol was added to the mixture. Approximately 2.5 g of anhydrous magnesium sulfate was added, followed by 25 mL of hexane/ethyl acetate solvent (90:10, v/v) containing 0.01% butylated hydroxytoluene (BHT). The mixture was homogenized using a Polytron® homogenizer for 1 min. After extraction, the mixture was centrifuged at 2220g for 10 min. The residue was repeatedly extracted using the same procedure. The combined filtrate was transferred to a 50 mL volumetric flask and massed up to volume of 50 mL with the extraction solvent. A 4 mL aliquot of the combined filtrates was evaporated with nitrogen gas, adjusted to the appropriate concentration of the assay samples using the mobile phase, and filtered through a 0.45 mm PTFE membrane filter (MSI Inc., Westboro, MA, USA). The normal-phase HPLC system (Jasco, HPLC PU-2089) consisted of a solvent delivery pump equipped with a fluorescence detector (Thermo Separation Products Inc., CA, USA) and a LiChrospher® Diol 100 column (250 × 4 mm, 5 μm). The isocratic mobile phase contained 1.1% isopropanol in *n*-hexane. The flow rate was 1.0 mL/min. The wavelengths were set at 290 nm for excitation and 330 nm for emission for identification and quantification of the tocopherols and tocotrienols. The tocopherol and tocotrienol peaks were identified by comparing their retention times to those of the standards (10–100 μg/mL).

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