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Phytochemicals and antioxidant capacity of natural food colorant prepared from black waxy rice bran

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ABSTRACT

This study determined the phytochemicals in black colorant powder (BCP) prepared from black waxy rice bran, including phenolic compounds, carotenoids, total anthocyanins, and antioxidant activity. The moisture content (MC) of the rice bran was adjusted to 30% and 40% (% wet basis). Four levels of electric field strength (E) of ohmic heating were applied to assist solvent extraction (OHM-ASE) to prepare the BCP. The steaming-assisted solvent extraction (ST-ASE) and solvent extraction (RB-SE) served as controls. BCP extraction using OHM-ASE with 30% MC (E=100, 150, and 200 V/cm) and 40% MC (E=50, 100, 150, and 200 V/cm) showed higher concentrations of gallic acid (253.29–257.57 μg/g), caffeic acid (129.34–136.12 μg/g) ferulic acid (630.74–663.34 μg/g), total phenolics (187.18–201.61 μg GAE/g), lutein (70.6–72.4 μg/g), β-carotene (4.81–5.12 μg/g), and total anthocyanins (812.17–847.09 mg/100 g) than those obtained from either ST-ASE or RB-SE. The zeaxanthin and *p*-coumaric acid concentrations in the BCP obtained from all OHM-ASE extracts were comparable to those from ST-ASE. In addition, the BCP extracted with OHM-ASE showed the strongest antioxidant activity of all treatments. This study demonstrated that BCP prepared from OHM-ASE can be considered as a valuable source of phytochemicals with high antioxidant properties.

1. Introduction

Black waxy rice (*Oryza sativa* L.), a special rice variety, has been cultivated and consumed for a long time in Asia, especially in Japan, Korea, China, and Southeast Asia. In Thailand, black waxy rice is commonly used as an ingredient in snack products or desserts, but not consumed as a main energy source (Tananuwong & Tewaruth, 2010). Recently, it has been recognized as a strong potential source of phytochemicals. In particular, the hydrophilic and semi-hydrophilic compounds, including the dark purple anthocyanins and polyphenols, which are distributed in the bran fraction (i.e. pericarp and aleurone layers) of the grain are of special interest (Abdel-Aal, Young, & Rabalski, 2006; Tananuwong & Tewaruth, 2010; Yawadio, Tanimori, & Morita, 2007). In addition to these hydrophilic phytochemicals, the black bran is an excellent source of bioactive lipophilic compounds such as tocopherols, tocotrienols, and γ-oryzanol (Qureshi, Sami, Salser, & Khan, 2002; Ryynänen, Lampi, Salo-Väänänen, Ollilainen, & Piironen, 2004). Carotenoids such as α-and β-carotene and zeaxanthin are also concentrated in the outermost pericarp, germ and aleurone layer of black rice bran (Pereira-Caro et al., 2013a; Pereira-Caro, Cros, Yokota, & Alan, 2013b). These compounds were shown to have positive health benefits for the prevention of chronic diseases related to oxidative stress and antioxidant activity (Hyun & Chung, 2004; Nam et al., 2006; Philpott, Gould, Lim, & Ferguson, 2004).

Currently, natural food coloring agents are in high demand by the food industry. This demand is as a result of not only their appearance and attractive consumer acceptability, but also because of their health benefits and safety aspects (Chou, Matsui, Misaki, & Matsuda, 2007). In the traditional extraction process, organic solvents such as absolute methanol, ethanol and acetone were widely used to extract the anthocyanin pigments and other important phytochemicals (Awika, Rooney, & Waniska, 2004; Cacace & Mazza, 2003; Kahkonen, Hopia, & Heinonen, 2001). Aqueous mixtures of these solvents were also commonly used (Pereira-Caro et al., 2013a,b; Tananuwong & Tewaruth, 2010). However, some disadvantages that limit their potential use in food manufacturing include relative instability of bioactive compounds and low extraction yields. Development of an extraction process that eliminates the problems, and possibly improves the yield and quality of the natural colorant is vital. Our previous studies indicated that rice bran oil extraction using ohmic heating improved the oil yield and bioactive compound content in the oil (Loypimai, Moonggarm, & Chottano, 2009; Loypimai, Moongngarm, & Chottanom, 2015a). Loypimai, Moongngarm, Chottanom, and Moontree (2015b) also showed that the preparation of natural colorant

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powder from black waxy rice bran using ohmic heating assisted by solvent extraction (OHM-ASE), contained high anthocyanins, tocols and γ-oryzanol. However, in addition to the predominant anthocyanins, tocols and γ-oryzanol, other phytochemicals such as phenolic compounds and carotenoids (β-carotene, zeaxanthin, and lutein) were also found in large quantities in black waxy rice bran. Therefore, OHM-ASE was applied to indicate that as well as containing high tocols and γ-oryzanol as shown in our previous study (Loypimai et al., 2015b), the natural colorant powder from black waxy rice bran was also high in phenolic compounds, carotenoids, and total anthocyanins as well as antioxidant activity compared with the composition of powder extracted using conventional methods.

2. Materials and methods

2.1. Chemicals and reagents

Standard (+)-catechin (PubChem CID: 24871278), *p*-coumaric acid (PubChem CID: 637542), syringic acid (PubChem CID: 10742), β-carotene (PubChem CID: 5280489), lutein and zeaxanthin (PubChem CID: 5280899), and maltodextrin (Dextrose equivalent (DE) 4-7) (Sigma-Aldrich Chemical Co., St. Louis, Mo, USA) were used as references along with HPLC grades of methanol, acetonitrile, and ethanol (BHD, Poole, UK). Standards of gallic, caffeic, and ferulic acids, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), vitamin C and Folin-Ciocalteu reagent were obtained from Fluka Chemical (Buchi, Switzerland). All chemicals and other reagents used were analytical grade.

2.2. Preparation of the bran sample

Black waxy rice (*Oryza sativa* L.), from special rice cultivars cultivated and consumed for a long time in Thailand, were purchased from a local rice factory in Roi Et Province, Thailand. The black waxy rice was milled using a local rice miller at the 10% degree of milling. After the milling process, the fresh bran was immediately passed through a 20 mesh sieve to remove the broken pieces of rice and husks. The chemical and bioactive components of the raw bran sample used for this study have been previously described.

2.3. Preparation of colorant powder (BCP) from black waxy rice bran

The BCP from black waxy rice bran was extracted using two methods as follows:

Ohmic heating (OHM-ASE) was applied to the black waxy rice bran to assist the subsequent solvent extraction of the BCP. The details of the procedure and the equipment used were similar to the method followed by our previous studies (Loypimai et al., 2009, 2015b). To summarize, the moisture content (MC) of the rice bran was adjusted to 30% and 40% (% wet basis) by adding deionized water. Then the bran samples were treated by OHM with four different levels of electrical field strength (E) of 50, 100, 150, and 200 V/cm, before solvent extraction. The ohmically-heated bran sample (20 g) was then extracted with 100 ml of acidified hydro-alcohol solution (water: 95% ethanol (1:1) acidified with 0.1 M HCl, pH 2.5), following the method reported by Duangmal, Saicheua, and Sueeprasan (2008) and Loypimai et al. (2015b). This bran extract was collected, added to maltodextrin which served as a combiner and stabilizer (2.0 g/100 ml of extract), mixed and frozen at −50 °C, before freeze-drying with a freeze dryer (FTS system Dura-Dry™, USA), at −50 °C condenser temperature for 20 h. The freeze-dried samples were weighed and ground into powder and passed through a 50 mesh sieve. The BCP samples were then stored in brown glass bottles (45 ml) and kept in a desiccator to avoid the moisture absorption until analysis.

Steaming-assisted solvent extraction (ST-ASE), without ohmic heating, followed the procedure of Juliano (1985). The rice bran sample (180 g) was steamed in an autoclave (ACV-3167 IWAKI) at 115 °C. When the inside temperature of the bran reached 105 °C it was held there for 1 min. The bran was then removed from the chamber, cooled to ambient temperature, and stored in a polyethylene bag. The steamed bran sample was extracted following the same procedure defined for the OHM-ASE treatment. The unheated (or raw rice bran) was also extracted using the same procedure and served as the control.

2.4. Scanning electron microscopy (SEM)

The rice bran meal samples obtained from OHM-ASE and ST-ASE were dried, fixed onto an aluminum sample holder and coated with gold before scanning using an SEM (JSM-6460L model, JEOL, USA) (Loypimai et al., 2015b).

2.5. Identification and qualification of phenolic compounds

2.5.1. Preparation of BCP extract

The BCP samples were extracted using a modification of the procedure described by Uzelac, Pospišil, Levaj, and Delonga (2005). A 0.5 g sample was added to 10 ml of methanol/HCl (100:1, v/v), which contained 2.0% butylated hydroxyanisole (BHA), then mixed and incubated in the dark at 35 °C for 12 h. The mixture was filtered through Whatman filter paper (no. 4), and stored in a brown glass vial overnight at 4 °C. The supernatant was then filtered through a 0.45 μm nylon syringe filter (Whatman, USA), and injected into the High Performance Liquid Chromatography (HPLC) system for the identification of the phenolic compounds. The extract preparation was performed in triplicate.

2.5.2. Determination of phenolic profiles using the RP-HPLC-DAD system

Analysis was conducted using an HPLC (Shimadzu LC-20AC pumps, SPD-M20A with a diode array detector (DAD)), and chromatographic separations were performed on a C_{18} Apollo (ø 4.6 × 250 mm, 5 μm, Alltech Associates, Deerfield, IL, USA), connected with an Inertsil ODS-3 guard column (ø 4.0 × 10 mm, 5 μm, GL Science Inc., Tokyo, Japan). The composition of solvents and the gradient elution conditions used followed the method of Butkhup and Samappito (2008) with slight modifications. The mobile system used was a gradient of solvent A containing acetonitrile/deionized water (2/97.8, v/v) containing 0.2% phosphoric acid, and solvent B which contained a mixture of acetonitrile/deionized water (97.8/2, v/v) containing 0.2% phosphoric acid, with a flow rate of 0.6 ml/min. The linear gradient started with 20% solvent B, 50% solvent B for 30 min, 60% solvent B for 35 min, and 20% solvent B for 40 min at isocratic elution until 55 min. Operating conditions were as follows: column temperature, 40 °C; injection volume, 20 μl; UV-diode array detection at 278 nm. The results were expressed as the amount of phenolic compounds in the colorant sample using the standard curve, compared with the retention time of the external standards.

2.5.3. Total phenolic content (TPC) assay

TPC was determined according to the method of Iqbal, Bhanger, and Anwar (2005). The reaction was initiated by mixing 0.2 ml of appropriate colorant extract, 0.8 ml of freshly prepared diluted Folin-Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (7.5%). The volume of the resulting mixture was adjusted to 7.0 ml with distilled water and then placed in the dark for 2 h to ensure completion of the reaction. The absorbance of the resulting blue-