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Ohmic heating-assisted extraction of anthocyanins from black rice bran to prepare a natural food colourant

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ABSTRACT

Black glutinous rice bran (*Oryza sativa* L.) is a potential source of the dark purple anthocyanin pigments. This study investigated some quality aspects and bioactive compounds of the colourant powder obtained from rice bran extracted by ohmic heating (OHM) assisted solvent extraction. The moisture content (MC) of the bran was adjusted to 30% and 40%. Four different levels of electric field strengths (E) of 50, 100, 150, and 200 V cm^{-1} were applied. The results showed that OHM assisted solvent extraction was a promising method offering both high yield and high concentration of bioactive compounds. The solubility, aw, bulk density, and color values (L^*, C^*, and h°) of the colorant powder of all treatments were comparable. The colourant powder obtained from the bran extracted using OHM with 30% MC (E=100, 150, and 200 V cm^{-1}) and 40% MC (E = 50, 100, 150, and V cm^{-1}) had the highest level of bioactive compounds.

Industrial relevance: The ohmic heating was a promising technique to assist solvent extraction of anthocyanin pigment from black rice bran. The colourant powder prepared by ohmic heating assisted (CP-OHM) had higher colourant yield, anthocyanin pigments, and bioactive compounds than conventional methods. The present study suggested that ohmic heating could be applied to develop industrial production scale of natural colourants.

1. Introduction

Currently, natural food colourant is in high demand by the food industry as a replacement for synthetic dye. Natural colourant is recognized for its optimistic health benefits and safety (Chou, Matsui, Misaki, & Matsuda, 2007). Anthocyanins are a group of plant pigments classified in the USA as natural food colourants under the category of fruits (21 CFR 73.250) and vegetables (21 CFR 73.260) (Lipman, 1996). In the EU they are classified under classification number E163 (Wrolstad, 2000). They are water-soluble glycosides and acylglycosides of anthocyanidins of various fruits, vegetables and cereal grains. Generally, they are found in the form of polyhydroxylated and methoxylated heterosides, which derive from the flavylium ion (2-phenylbenzopyrilium in nature) (Castañeda-Ovando, Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Wu, Gu, Prior, & McKay, 2004). They have been recognized as health-enhancing substances attributable to their antioxidant, anti-inflammatory and hypoglycemic effects (Nam et al., 2006; Philpott, Gould, Lim, & Ferguson, 2004) as well as other biological effects, including antimutagenic and anticarcinogenic activities (Hyun & Chung, 2004; Nam, Choi, Kang, Kozukue, & Friedman, 2005). There are numerous naturally occurring anthocyanins; however, only six anthocyanidins, namely pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin, are widespread in fruits and cereal grains (Escribano-Bailon, Santos-Buelga, & Rivas-Gonzalo, 2004).

Black glutinous (waxy) rice bran (*Oryza sativa* L.) is a potential plant source of dark purple anthocyanin pigment. In general, pigments are distributed in the aleurone and pericarp layers, which are removed from the rice grain into the bran fraction during the milling process (Tananuwong & Tewaruth, 2010; Yawadio, Tanimori, & Morita, 2007). It contains approximately 3.31 mg/g of anthocyanin in the bran (Nontasan, Moongngarm, & Deeseenthum, 2012). Moreover, the bran fraction is an excellent source of bioactive compounds including tocopherols, tocotrienols, γ-oryzanol and phenolic compounds (Qureshi, Sami, Salser, & Khan, 2002; Ryynänen, Lampi, Salo-Väänänen, Ollilainen, & Piironen, 2004). Traditionally, organic solvents such as absolute methanol, ethanol and acetone have been used to extract the anthocyanin colour (Awika, Rooney, & Waniska, 2004; Cacace & Mazza, 2003; Kahkonen, Hopia, & Heinonen, 2001). Aqueous mixtures of solvents with small amounts of acid have also been used (Duangmal, Saicheua, & Sueeprasan, 2008; Tananuwong & Tewaruth, 2010). The extraction processes used at the present time are limited because of their relative instability and low extraction percentages. An extraction method capable of obtaining high concentrations of anthocyanins and other functional substances, as well as colour yield, needs to be advanced.

Ohmic heating (OHM) is one of the most promising methods of extracting anthocyanin colour from rice bran. OHM, also known as electroconductive heating, utilizes the inherent electrical resistance of

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food materials to generate heat (Wang et al., 2007). Most food materials contain ionic constituents, such as salts and acids, allowing the conduction of electrical current (Palaniappan & Sastry, 1991). This process can be used to generate heat within the product, transforming the electrical energy into thermal energy and thus heating materials at exceptionally rapid rates without the need for a heating medium or surface. This process avoids excessive thermal damage to labile substances, such as vitamins and pigments (Castro, Teixeira, Salengke, Sastry, & Vicente, 2004; Palaniappan & Sastry, 1991; Sastry & Barach, 2000). The success of actual and potential applications for OHM can be found in several processes, including blanching, evaporation, dehydration, pasteurization and extraction (FDA, 2000). In particular, the extraction process has been used to increase the efficiency of solute diffusion throughout the membrane (electro-osmosis effect), resulting in a better-quality product (Boussetta, Lanoisellé, Bedel-Cloutour, & Vorobiev, 2009; Donsì, Ferrari, Fruilo, & Pataro, 2010; Puértolas, Hernandez-Orte, Sladana, Alvarez, & Raso, 2010). More studies have found that OHM has been shown to increase the extraction yields of sucrose from sugar beets (Katrokha, Matvienko, Vorona, Kupchik, & Zaets, 1984), apple juice from apples (Lima & Sastry, 1999), beet dye from beetroot (Halden, de Alwis, & Fryer, 1990; Lima, Heskett, & Sastry, 1999; Schreier, Reid, & Fryer, 1993), rice bran oil and bioactive substances from rice bran (Lakkakula, Lima, & Walker, 2004; Loypimai, Moonggarm, & Chottanom, 2009), and polyphenols from red grape pomace (Darra, Grimi, Vorobiev, Louka, & Maroun, 2013); however, no investigation has been conducted on the use of OHM to extract food colourant from black rice bran. This study was carried out to investigate the physical quality, bioactive and anthocyanin contents of colourant powder obtained from black rice bran using OHM to assist the solvent extraction.

2. Materials and methods

2.1. Chemicals and reagents

Standards of cyanidin-3-*O*-glucoside chloride (PubChem CID:197081), malvin (PubChem CID:5458954), delphinidin (PubChem CID:68245), pelargonidin (PubChem CID:67249), cyanidin (PubChem CID:68247), malvidin (PubChem CID:69512), cyanidin-3-*O*-rutinoside chloride (PubChem CID:16212330), (±)-α-tocopherol (PubChem CID:14985), (+)-δ-tocopherol (PubChem CID:92094), (+)-γ-tocopherol (PubChem CID:92729), (+)-γ-tocotrienol (PubChem CID:5282349) and maltodextrin (Dextrose equivalent (DE) 4–7) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). γ-Oryzanol (PubChem CID: 5282164) standard was purchased from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan). HPLC grades of methanol, acetonitrile, hexane, n-butanol and ethanol were purchased from BHD (Poole, UK). All chemicals and reagents were analytical grade.

2.2. Rice bran preparation

Black glutinous rice bran (*O. sativa* L.) samples were purchased from a rice milling factory (10% degree of milling) in Roi Et province, Thailand. The fresh bran samples were prepared in triplicate. In total there were 30 samples for 10 treatments including the raw bran. All samples were immediately passed through a sieve (no. 20) to remove broken pieces of rice and husks to obtain the rice bran fraction. The initial moisture content of the rice bran was determined using the AOAC (2000) method.

2.3. Scanning electron microscopy (SEM)

The raw, ohmically treated and steamed bran samples were dried before fixing them on an aluminium sample holder. All the samples were gold coated and scanned using a scanning electron microscope (SEM) (JSM-6460 L model, JEOL, USA) under high-vacuum condition with an accelerating voltage of 10.0 kV and at a working distance of 30.0 mm.

2.4. Preparation of colourant powder

Two extraction processes were applied to prepare the natural food colourant from black glutinous rice bran: (1) rice bran was steamed and extracted by solvent and (2) rice bran samples were treated using OHM (Sang Chai Meter Co. Ltd., Thailand) with two levels of moisture content (MC 30% and 40%). The details of the equipment were similar to that indicated by Loypimai et al. (2009) with some modifications. Briefly, a sample of 180 g of rice bran was placed in a chamber (size 14.4 × 14.4 × l.5 cm) between titanium electrodes (size 40 × 30 mm), connected to a dielectric ohmic heater and enclosed in a Teflon tee (Fig. 1). Then, an alternating current of 50 Hz with four different levels of electrical field strengths (E) of 50, 100, 150 and 200 V cm^{-1} was applied. Raw rice bran was also directly extracted by solvent without being heated. There were 10 treatments in this study.

2.4.1. Preparation of colourant powder using ohmic heating-assisted solvent extraction (CP-OHM)

The rice bran was added with deionized water to adjust its moisture content (MC) to 30% and 40% (%wet basis). The bran sample was heated by OHM according to the method described previously by Loypimai et al. (2009). During OHM, the voltage, current and temperature were measured continuously using a data logger controller (Digicon, DP-74SD). When the temperature of the heated bran inside the chamber reached 105 °C, it was held there for a minute and then immediately removed from the chamber. It was allowed to cool to room temperature. The heated sample was extracted according to the method reported by Duangmal et al. (2008) with small modifications. A sample of 20 g was extracted with 100 ml of acidified hydroalcoholic solution (water: 95% ethanol; 1:1 and acidified with 0.1 M HCL to obtain a pH of 2.5). The bran and solution were mixed with a vortex mixer (VELP Scientifica, Europe) for a minute and then placed in an orbital shaker (Gerhardt LS500, UK) at 100 rpm for 90 min. The slurry was filtered through a V-700 vacuum pump (Buchi, Switzerland) with a filter paper (Whatman No. 4). The extract was added with maltodextrin (2 g/100 ml of extract) and frozen at −50 °C before freeze-drying with a freeze dryer (FTS system Dura-Dry™, USA) under 200–250 mT vacuum, at −50 °C condenser temperature for 20 h. The dried colour was weighed, ground into powder and passed through a 50-mesh sieve. The colourant powder was obtained and kept in a brown glass bottle (45 ml). The CP-OHM samples were placed in desiccators and stored at 4 °C until the analysis for physical properties and chemical compositions.

2.4.2. Preparation of colourant powder using steaming-assisted solvent extraction (CP-ST)

Steam heating was conducted by following the method developed by Juliano (1985). A 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 for a minute, removed from the chamber and cooled at an ambient temperature. To obtain the CP-ST and CP-RB (colourant from raw rice bran), the steamed and raw bran samples were extracted, dried, stored and analysed using the same conditions as the CP-OHM.

2.5. Chemical analysis

Moisture content, crude protein, ash, crude fat and total dietary fibre of the raw rice bran samples were determined according to the official methods of AOAC (2000) in triplicate. The water activity (a_w) of the colourant powder was measured with a digital a_w meter (Aqualab®, USA).