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Simultaneous quantification of vitamin E, γ-oryzanols and xanthophylls from rice bran essences extracted by supercritical CO_2

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ABSTRACT

Since the nutrition value of rice is diminished during rice processing, technology that can preserve and sustain functional compounds is necessary. In this study, supercritical carbon dioxide (SC-CO_2) extraction was optimized for operational conditions (time, temperature, pressure and modifier) to extract vitamin E, γ-oryzanols and xanthophylls from rice bran. The simultaneous quantification of the compounds was developed using high-performance liquid chromatography with diode array and fluorescence detectors. Central composite design and respond surface methodology were applied to achieve optimum extraction conditions. The optimized conditions were 60 min, 43 °C, 5420 psi with 10% ethanol as a modifier. Pigmented rice bran extracts contained greater amounts of functional phytochemicals than non-pigmented rice bran extracts (0.68, 1410, and non-detectable μg/g compared with 16.65, 2480, and 0.10 μg/g of vitamin E, γ-oryzanols and xanthophylls in pigmented and non-pigmented ones, respectively). SC-CO_2 extraction with modifier would be promising for preparation of phytochemical essences for therapeutic purpose.

1. Introduction

Rice bran and others such as wheat bran are abundant sources of functional compounds including vitamin E (tocopherols and tocotrienols), γ-oryzanols, carotenoids, phenolic compounds, and unsaturated fatty acids (Durante, Lenucci, Rescio, Mita, & Caretto, 2012; Sookwong, Nakagawa, Murata, Kojima, & Miyazawa, 2007; Zhang, Zhang, Zhang, & Liu, 2010). Physiological advantages of these functional compounds have been evidenced as anti-oxidative, anti-hypercholesterolemic, neuroprotective, and anti-angiogenic properties (Khanna et al., 2005; Miyazawa et al., 2009; Qureshi, Sami, Salser, & Khan, 2002; Serbinova, Kagan, Han, & Packe, 1991). However, despite these beneficial effects, consumers hardly receive these compounds on a daily basis. For instance, rice bran, the main source of the compounds, is removed during the rice polishing process that makes commercial white rice. At present, rice bran oil supplements have been recommended as a good source of these bioactive compounds. Unfortunately, due to thermal processes of oil manufacturing, some bioactive compounds either deteriorate or separate into inedible waste (e.g., vitamin E is concentrated in deodorized rice bran scum oil but not in commercial rice bran oil) (Bruscatto et al., 2009). Therefore, in order to achieve the most health benefit of rice bran, technology that can sustain the nutritive value and functional compounds of rice bran should be considered.

Supercritical carbon dioxide (SC-CO_2) extraction technology is a green procedure for extracting natural products from plants samples, and the technology has gained increasing interest in the food, pharmaceutical, and cosmetic industries (Chen & Ling, 2000; Ferreira, Nikolov, Doraiswamy, Meireles, & Petenatee, 1999). SC-CO_2 extraction technology has the advantages of being non-flammable, no toxic, cost-effective and easily removed from the extract following decompression (Chiappini, Perraudin, Durand-Jolibois, & Doussin, 2006). Recently, SC-CO_2 extraction

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has shown a promising potential for extraction of rice bran functional compounds (Sarmento, Ferreira, & Hense, 2006; Wang et al., 2008). For instance, the concentration of γ-oryzanols in extracted rice bran oil was greater than in rice bran oil derived by hexane Soxhlet extraction (Wang et al., 2008). Rice bran oil extracted by using SC-CO_2 was reported to be enriched in tocopherols and tocotrienols, suggesting the oil to be a good source of natural vitamin E (Sarmento et al., 2006). However, these reports concerned extraction conditions that were not designed for the various groups of rice bran functional compounds, such as vitamin E, γ-oryzanols, and xanthophylls. Hence, there is a need to develop a method to simultaneously extract and analyze these compounds.

In this study, supercritical CO_2 was used to extract nutritive essences, including vitamin E, γ-oryzanols and xanthophylls from rice bran samples. To our knowledge, this may have been the first time that the simultaneous analysis of rice bran vitamin E, γ-oryzanols and xanthophylls was developed using high-performance liquid chromatography. The extraction parameters and the influence of modifiers were evaluated using response surface methodology (RSM) with central composite design (CCD). This expanded knowledge concerning the application of SC-CO_2 extraction would be useful to increase the use of the technology in the food and pharmaceutical industries.

2. Material and methods

2.1. Plant materials

Eight bran samples of rice (*Oryza sativa* L.) from ordinary non-glutinous rice varieties Hawm Phitsanulok 1 (HPSL, a variety with high production and good eating quality) and RD41 (a variety with high production yield and insect resistance), ordinary glutinous rice varieties RD6 (very popular Thai glutinous rice variety) and Sew Mae Jan (SMJ, a variety with good eating quality), pigmented non-glutinous rice varieties Hua Bon (HB, a variety popular in Southern Thailand), Riceberry (RB, a very popular variety) and Hom Payom (HPY, a variety with high production and drought resistance) and a pigmented glutinous rice variety Leum Pua (LP, a very popular variety with good eating quality) were provided from the Phitsanulok Rice Research Center in Phitsanulok province, Thailand in 2013. The fresh rice paddies were harvested and sun dried until the moisture content was reduced to below 14%. Rice grains were dehusked and milled by a local milling machinery (Natrawee Technology, Chachoengsao, Thailand). The bran samples were sieved through 45 mesh screen, sealed in a plastic bag under reduced pressure, and kept in a cold room (4 °C) until experimentation.

2.2. Supercritical fluid extraction

Supercritical CO_2 extraction was carried out using SFX™ 220 (ISCO, Lincoln, NE, USA). Pure CO_2 was applied by using a syringe pump (ISCO Medel 100DX, USA). Rice bran sample (4.00 g) was filled in a sample cell, and extracted supercritically with CO_2. Optimized operational parameters were extraction time, temperature, pressure, and modifier. Among these parameters, extraction time (10, 20, 30, 45, 60, 75, and 90 min) was optimized first and judged based on extraction efficiency and operation performance. Temperature (40, 55, and 70 °C), pressure (3000, 5000, 7000 psi) were optimized using experimental design because they are highly important parameters for extraction. After this, the influence of additional modifiers (methanol, ethanol, and isopropanol at the concentration of 1, 5 and 10% (v/v)) was investigated. The extract was trapped by bubbling the CO_2 eluent in a glass tube containing dichloromethane/ethanol (2:1, v/v, 10.0 mL). After extraction, the solutions were concentrated using a rotary evaporator (Buchi, R-124, Switzerland). Following this, the extracts were analyzed by high-performance liquid chromatography with diode array detector and fluorescence detector (HPLC-DAD-FLD) to determine the content of vitamin E, γ-oryzanols and xanthophylls.

2.3. Chemicals

The dichloromethane, methanol, ethanol, and isopropanol used were purchased from Merck (Germany). Regarding standard vitamin E, four isoforms (α-tocopherol, α-tocotrineol, γ-tocotrienol, and δ-tocotrienol) were from Sigma (USA), and three isoforms (β-tocopherol, γ-tocopherol, and δ-tocopherol) were from Eisai Food & Chemical Co. Ltd. (Japan). Standard γ-oryzanols (98%, Ichimaru Pharcos, Japan) were kindly provided by Prof. Dr. Malyn Chulasiri, and standard xanthophyll (lutein) was obtained from Chengdu Biopurify Phytochemicals (China).

2.4. Experimental design for extraction of rice bran samples

The optimum extraction temperature and extraction pressure for SC-CO_2 extraction were determined using response surface methodology (RSM) with a three-level and two-factor central composite design (CCD). Two factors of extraction temperatures (X_1) and pressures (X_2) were chosen as independent variables. The coded and real values of temperature (X_1) were −1 for 40 °C, 0 for 55 °C, and +1 for 70 °C, and those of pressure (X_2) were −1 for 3000 psi, 0 for 5000 psi, and +1 for 7000 psi. Three responses of total vitamin E (Y_1), total γ-oryzanols (Y_2) and total xanthophylls (Y_3) were selected as the dependent variables. Regression analysis was done on the data obtained by triplicate analysis for each dependent variable using Minitab statistical software (Trial version 16.01, Minitab Inc., State College, PA, USA). Response surface analysis was also applied to the data from CCD for modeling and prediction of optimum extraction temperature and extraction pressure for total vitamin E, total γ-oryzanol and total xanthophyll contents from the rice bran.

The fitting was done using a second-order model for each response. This model was expressed with the coded variables (X_1 and X_2) with the following equation:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_1X_2 + \varepsilon$$

where Y represents the estimated response, B_0 represents the equation parameters for the constant term, B_1 and B_2 represents the linear coefficients, B_{11} and B_{22} represents the quadratic coefficients, B_{12} represents the interaction coefficients, and ε the random error.

2.5. Simultaneous analysis of vitamin E, γ-oryzanols, and xanthophylls using HPLC-DAD-FLD

Quantitative analysis of vitamin E, γ-oryzanols and xanthophylls was operated simultaneously using a HPLC-DAD-FLD system consisting of an Agilent HPLC 1100 connected to a diode array detector (Model G1315 A, Agilent Technologies, Palo Alto) and a fluorescence detector (Model 1046A, Hewlett Packard, USA). The separation was achieved using a Halo C18 (2.1 × 10 mm, 2.7 μm, Advanced Materials Technology Inc., DE, USA) at 40 °C. The gradient profile was 0–30 min, 80–100% methanol linear gradient, and 30–70 min, 100% methanol. The flow rate was maintained at 0.15 mL/min. Vitamin E was fluorescently detected using excitation wavelength of 294 nm and emission wavelength of 326 nm. γ-Oryzanols and xanthophylls were detected using diode array detection at 330 and 450 nm, respectively. Their quantitative determinations were calculated using standard calibration curves