Food Chemistry 141 (2013) 2821–2827

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Phytochemical profile of a Japanese black–purple rice

Gema Pereira-Caro [a], Shin Watanabe [b], Alan Crozier [a], Tatsuhito Fujimura [c], Takao Yokota [d], Hiroshi Ashihara [b,e,*]

[a] Joseph Black Building, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom
[b] Iriomote Station, Tropical Biosphere Research Center, University of Ryukyus, 870, Uehara, Taketomi-cho, Yaeyama-gun, Okinawa 907-1541, Japan
[c] Faculty of Life and Environmental Sciences, University of Tsukuba, Tennoudai 1-1-1, Tsukuba 305-8572, Ibaraki, Japan
[d] Department of Biosciences, Teikyo University, Utsunomiya 320-8551, Japan
[e] Department of Biological Sciences, Graduate School of Humanities and Science, Ochanomizu University, Bunkyo-ku, Tokyo 112-8610, Japan

ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 21 May 2013
Accepted 23 May 2013
Available online 31 May 2013

Keywords:
Japanese black–purple rice
Anthocyanins
Flavones
Flavonols
Carotenoids
γ-Oryzanol

ABSTRACT

Black–purple rice is becoming popular with health conscious food consumers. In the present study, the secondary metabolites in dehulled black–purple rice cv. Asamurasaki were analysed using HPLC–PDA–MS^2. The seeds contained a high concentration of seven anthocyanins (1400 μg/g fresh weight) with cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside predominating. Five flavonol glycosides, principally quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside, and flavones were detected at a total concentration of 189 μg/g. The seeds also contained 3.9 μg/g of carotenoids consisting of lutein, zeaxanthin, lycopene and β-carotene. γ-Oryzanol (279 μg/g) was also present as a mixture of 24-methylenecycloartenol ferulate, campesterol ferulate, cycloartenol ferulate and β-sitosterol ferulate. No procyanidins were detected in this variety of black–purple rice. The results demonstrate that the black–purple rice in the dehulled form in which it is consumed by humans contains a rich heterogeneous mixture of phytochemicals which may provide a basis for the potential health benefits, and highlights the possible use of the rice as functional food.

1. Introduction

Black–purple rice known as Kokushimai is the commercial name of coloured cultivars of *Oryza sativa* sold in Japan. The cultivar, Asamurasaki is derived from the hybrid of Japanese sticky rice and Bali black rice which was bred by the NARO Tohoku Agricultural Research Center and registered in 1996. The consumption of black rice is becoming popular in Japan and other Asian countries where it is often mixed with white rice prior to cooking to enhance the quality and flavour. Although on a small scale, it is also used to make red saké.

Several studies have suggested an association between rice intake and health effects which are attributed to its high amounts of fibre and phytochemicals such as tocopherols, tocotrienols, vitamin B complex, vitamin E complex, γ-oryzanols and phenolic compounds (Irakli, Samanidou, Biliaderis, & Papadoyannis, 2012; Lee, Kim, Koh, & Ryu, 2006). There are also reports on extracts of black rice bran (i.e., the husks of the seed) suggesting beneficial effects on health (Choi, Kim, & Friedman, 2013; Leardkamolkarn Thongthep, Suttiarporn, Kongkachuichai, Wongpornchai and Wanavijitr, 2011). The main secondary metabolites in black rice are the anthocyanins, cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside which are localised in the pericarp and aleurone layers of the seeds (Abdel-Aal, Young, & Rabalski, 2006). Intake of black rice anthocyanins by dyslypidemic rats on a high fat diet has been shown to reduce platelet hyperactivity, hyperglyceridemia and facilitate the maintenance of optimal platelet function (Yang et al., 2011). However, black rice contains other secondary metabolites in addition to anthocyanins. Recently, Sriseadka, Wongpornchai, and Rayanakorn (2012) identified eleven flavonoids and their glycosides in bran extracts of seven varieties of Thai black rice. Frank, Reichardt, Shu, and Engel (2012) reported on a comparative capillary gas chromatography-based metabolite profiling of coloured rice grains. A broad spectrum of lipophilic and hydrophilic low molecular weight constituents from different chemical classes were detected, but individual metabolites were not identified.

The present study identified and quantified a variety of anthocyanins, flavones and flavonols, carotenoids and γ-oryzanol in dehulled Japanese black–purple rice seed using an integrated approach consisting of HPLC with a photodiode array detector (PDA) and electrospray ionisation tandem mass spectrometry (MS^2). The overall characterization of secondary metabolites in black–purple rice gives more detail to support the rice as an effective health promoting food.

* Corresponding author at: Department of Biological Sciences, Graduate School of Humanities and Science, Ochanomizu University, Bunkyo-ku, Tokyo 112-8610, Japan. Tel./fax: +81 5700 4225.
E-mail address: ashihara.hiroshi@ocha.ac.jp (H. Ashihara).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.05.100

2. Experimental

2.1. Plant material

Field grown, seeds of black–purple rice (*O. sativa* cv. Asamurasaki), were harvested in 2012 on Iriomote Island, Okinawa, Japan after which they were dehulled.

2.2. Chemicals

Cyanidin-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside were purchased from extrasynthese (Lyon, France). Pelargonidin-3-*O*-glucoside and peonidin-3-*O*-glucoside were supplied by PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Acetonitrile, acetone and methanol were purchased from Fisher Scientific Ltd. (Loughborough, Leicestershire, UK). β-Carotene, lutein, hexane, formic acid, acetic acid and methyl *tert*-butyl ether (MTBE) were supplied by Sigma (Poole, UK). γ-Oryzanol was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

2.3. Extraction of anthocyanins, flavones and flavonoids

Two grams of rice seeds were soaked in 60 mL of water/acetic acid (99:1, v/v) for 12 h. The acidified water was then passed through an SPE cartridge, (Strata C18, 55 μm, 70 Å, 10 g) (Phenomenex, Macclesfield, Cheshire, UK) previously pre-conditioned with 6 mL of methanol, followed by 6 mL of water/formic acid (99:1, v/v), after which it was eluted with 6 mL of methanol/formic acid (99:1, v/v). Rice samples were homogenised with 10 mL of 1% formic acid in methanol using an Ultra-Turrax homogenizer (T25 basic, IKA Werke KG, Staufen, Germany) for 2 min at 24,000 rpm prior to being centrifuged for 15 min at 4000×*g*. The pellet was re-extracted three times with 10 mL of the same solvent. The SPE methanolic eluate and all supernatants were pooled before being reduced to dryness *in vacuo* at 30 °C. The residues were re-suspended in 5 mL of methanol/formic acid (99:1, v/v) and anthocyanins and other flavonoids were analysed by HPLC–PDA–MS^2 in triplicate.

2.4. Extraction of procyanidins

For procyanidins analysis with a degree of polymerization >2, the method of Robbins et al. (2009) was used. Briefly, 2 g of rice seeds were homogenised with 5 mL of acetone/water/acetic acid, (70:29.5:0.5, v/v/v). Samples were centrifuged at 3000×*g* for 15 min at 4 °C and supernatants were collected. The pellet was re-extracted three times with 5 mL of the same solvent as described above. All supernatants were pooled and passed through a SPE cartridge, Strata SCX (55 μm, 70 Å, 500 mg/3 mL) (Phenomenex, Cheshire, UK), following preconditioning of the cartridge with distilled water. 5 mL of the cartridge eluate was analysed in triplicate by HPLC with photodiode array, fluorescence and mass spectrometric detection (HPLC–PDA–FL–MS^2).

2.5. Extraction of carotenoids and γ-oryzanol

The method of Li et al. (2011) was adapted to extract carotenoids and γ-oryzanol from rice seeds. 2 g of rice seeds were homogenised with 10 mL of ethanol/hexane (4:3, v/v) using an Ultra-Turrax homogenizer for 2 min at 12,000 rpm, prior to being centrifuged for 15 min at 3500×*g*. The pellet was re-extracted twice with 10 mL of hexane, and centrifuged. The supernatant were pooled and washed first with 10 mL distilled water and then with 5 mL of a 10% aqueous NaCl solution. The organic phase was retained and reduced to dryness under a gentle stream of nitrogen, before being dissolved in 500 μL MTBE/methanol (90:10, v/v). All procedures were performed quickly avoiding exposure to light, oxygen, high temperature and also pro-oxidant metals. Aliquots of carotenoids were analysed in triplicate by HPLC–PDA–MS^2 and γ-oryzanol by HPLC–PDA.

2.6. Qualitative and quantitative analysis by HPLC–PDA–MS^2

Rice seed extracts were analysed on a Surveyor HPLC system equipped with a PDA detector scanning from 200 to 600 nm, and an autosampler (Thermo Finnigan, San Jose, CA) cooled at 4 °C. Separation of anthocyanins and other secondary metabolites from rice seeds were performed using a Synergi 4 μm Max-RP 80 Å 250 × 4.6 mm i.d. reverse phase column (Phenomenex) maintained at 40 °C and preceded by a C12 (4 × 3.0 mm i.d.) precolumn (Phenomenex). The mobile phase, pumped at a flow rate of 1 mL/min, was: (i) a 60 min, 10–40% gradient of methanol in 1% aqueous formic acid for the analysis of anthocyanins; and (ii) a 75 min, 10–40% gradient for 60 min, followed by a 15 min 40–50% gradient of methanol in 1% aqueous formic acid for the analysis of flavones and flavonols.

The analysis of procyanidins was carried out using the HPLC system with a fluorescence detector (FP-920, Jasco (UK) Ltd.) and a mass spectrometer. The separation was achieved using a Develosil Diol 100 Å (250 × 4.6 mm, 5 μm) column (Phenomenex, Cheshire, UK) with chromatographic conditions that has been previously described by Robbins et al. (2009).

Analysis of carotenoids and γ-oryzanol was carried out on a Kinetex XB-C18 column (2.6 μm, 150 × 4.6 mm i.d.) with a C18 (4.0 × 3.0 mm i.d.) precolumn (Phenomenex) maintained at 35 °C and eluted isocratically with methanol/MTBE/water (85:14:0.5, v/v/v) at a flow rate of 1 mL/min. After passing through the flow cell of the diode array or the fluorescence detector, the column eluate was split and 0.3 mL/min was directed to either an LCQAdvantage ion trap mass spectrometer fitted an electrospray interface (ESI) operating in positive ionisation mode for anthocyanins and in negative ionisation mode for flavone, flavonols and γ-oryzanols; or to an LCQDecaXP ion trap mass spectrometer fitted with ESI operating in positive ionisation mode for carotenoids; or an LCQDuo IT mass spectrometer fitted with an electrospray interface operating in negative ionisation mode for procyanidins.

Identification of anthocyanins, flavones and flavonols in all samples were carried out using full scan, data-dependent MS^2 scanning from 100 to 1000 *m*/*z* and selected reaction monitoring. With ESI in positive ionisation mode, capillary temperature was 300 °C, sheath gas was 50 U, auxiliary gas was 40 U, and source voltage was 3 kV for anthocyanins; while for carotenoids, capillary temperature was set to 325 °C, sheath gas flow was 40 U, auxiliary gas was 20 U, and source voltage was 4.1 kV. For negative ionisation, capillary temperature was set to 300 °C, sheath and auxiliary gas were 70 and 60 U, respectively, and source voltage was 5 kV.

Anthocyanins in rice seeds were quantified on the basis of chromatographic peak areas acquired at 520 nm and expressed as cyanidin-3-*O*-glucoside equivalents while the quantification of flavones and flavonols were based on the absorbance response at 365 nm and expressed as available commercial standards or quercetin equivalents. Carotenoids were identified according to the following criteria: retention times and absorbance spectra of commercially available standards or absorbance spectra compared with data available in literature. For identification proposes, full scan data acquisition (*m*/*z* 300–800) and selective ion monitoring were carried out. The carotenoids were quantified from their chromatographic peak areas acquired at 450 nm. Lutein was quantified by reference to a standard while β-carotene, zeaxanthin and lycopene were quantified in β-carotene equivalents.