Food Chemistry 217 (2017) 98–105

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of post-harvest treatment on bioactive phytochemicals of Thai black rice

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 19 August 2016
Accepted 23 August 2016
Available online 24 August 2016

Chemical compounds studied in this article:
Cyanidin-3-*O*-glucoside (PubChem CID: 441667)
Peonidin-3-*O*-glucoside (PubChem CID: 443654)
Cycloartenyl ferulate (PubChem CID: 59271038)
Campesterol ferulate (PubChem CID: 91746242)
α-Tocopherol (PubChem CID: 14985)
γ-Tocotrienol (PubChem CID: 5282349)

Keywords:
2-Acetyl-1-pyrroline
Anthocyanins
Black rice
Drying
γ-Oryzanols
Packaging
Storage
Vitamin E

ABSTRACT

Because black rice is rich in antioxidants, appropriate methods of post-harvest treatment are necessary for maintaining these bioactive phytochemicals. Drying methods, storage temperatures, storage duration, and packaging methods affected the contents of some bioactive compounds in the two varieties of Thai black rice used in this research. Sun drying reduces the loss of anthocyanins and γ-oryzanols more than does hot air drying. Glutinous black rice stored as paddy at cool room temperature retains more anthocyanins, γ-oryzanols, and vitamin E than does paddy stored at room temperature. Nylon/LLDPE pouches containing N_2 are the most suitable packaging for preserving the key aroma compound 2-acetyl-1-pyrroline (2AP), total phenolic, and anthocyanin contents of unpolished aromatic black rice. These pouches also retard the formation of some common off-flavor compounds.

1. Introduction

Black rice has gained increasing popularity today as a staple food and is replacing white rice, due to its health-promoting and chronic-disease-preventing properties (Chiang et al., 2006; Wang et al., 2007). The health benefits of black rice are attributed to the bioactive pigments located in the bran layer of the rice. Apart from γ-oryzanols and vitamin E, there are some water-soluble pigments responsible for bran color and anti-oxidative property of black rice. Two major anthocyanins found in black rice are cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside (Hou, Qin, Zhang, Cui, & Ren, 2013).

Two types of Thai black rice (*Oryza sativa* L.) are available in the rice market: glutinous and non-glutinous. Black glutinous rice is a native variety widely grown in northern Thailand and is mostly consumed in a sweetened form or as a dessert. However, consumption of black glutinous rice cv. Luem Pua (LP) as a staple food is becoming increasingly popular because of its better nutritional, aroma, and textural properties. Likewise, black non-glutinous rice, cv. Jao Hom Nin (JHN), which means black gem jasmine rice, is a selected line of Khao Dawk Mali 105, the most popular non-glutinous Thai jasmine rice variety.

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http://dx.doi.org/10.1016/j.foodchem.2016.08.084
0308-8146/

The JHN black rice has a distinctive pleasant aroma and is becoming a popular alternative to white non-glutinous rice. The *N*-heterocyclic compound 2-acetyl-1-pyrroline (2AP) is considered a key aroma-active compound of aromatic rice. It plays an important role in the sensory quality of rice, since its concentration assures rice aroma quality. The high concentration of 2AP in some aromatic white rice varieties, such as Khao Dawk Mali 105 and Basmati, is conducive to their high favor and price.

There is evidence that the stability of bioactive phytochemicals in rice can be affected by some post-harvest treatments, including drying (Wongpornchai, Dumri, Jongkaewwattana, & Siri, 2004) and packaging methods (Tananuwong & Tangsrianugul, 2013), storage temperature, and storage duration (Zhou, Chen, Zhang, & Blanchard, 2014). Moreover, changes in rice aroma that may be both desirable and undesirable are also induced by post-harvest treatments. Our previous study (Wongpornchai et al., 2004) found that 2AP content and the milling quality of the aromatic white rice cv. Khao Dawk Mali 105 dried by hot air at the relatively low temperatures of 30 °C and 40 °C were better preserved in comparison with hot air drying at 70 °C. In the same way, suitable post-harvest management should lower the reduction rate of black rice phytochemicals. Several studies concerning the bioactive phytochemicals in pigmented rice have been made to provide useful information especially for the development and selection of phytochemical-rich genotypes (Shen, Jin, Xiao, Lu, & Bao, 2009; Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011; Zhang, Zhang, Zhang, & Liu, 2010). However, few studies have reported the effects of post-harvest treatment on the content of black rice anti-oxidants, especially those that possess biological activities beneficial to human health, such as anthocyanins (Htwe et al., 2010b), γ-oryzanols, and vitamin E (Pascual et al., 2013).

This research was done to determine appropriate drying, packaging, and storage conditions for preserving the bioactive phytochemicals of black rice during storage. LP is a Thai black glutinous rice that contains more anthocyanins than some other Thai black rice varieties. Since LP rice is now in greater demand as a source of healthy food products, it was used in this study to investigate the effects of drying methods and storage conditions on the contents of anthocyanins, γ-oryzanols, and vitamin E in this rice. To determine the effects of packaging methods and storage time, the 2AP, off-flavor compound, total phenolic and anthocyanin contents of JHN, the popular Thai black non-glutinous rice, were investigated.

2. Materials and methods

2.1. Rice samples

Seeds of Thai black non-glutinous JHN rice were harvested in the experimental field of Kasetsart University, Kamphaengsaen Campus, Nakorn Pathom province in central Thailand in 2012. Seeds of the Thai black glutinous LP rice were harvested and provided by the Phitsanulok Rice Research Center, in Phitsanulok province in northern Thailand in 2013. LP rice seeds were de-hulled using a laboratory rice husker (TR-200; Kett, Tokyo, Japan) just before each analysis of phytochemical contents.

2.2. Materials and chemicals

Cyanidin-3-*O*-glucoside and naringin were obtained from Sigma-Aldrich Chemicals (St. Louis, MO). γ-Oryzanols, Folin-Ciocalteau phenol reagent, gallic acid, and anhydrous sodium carbonate (Na_2CO_3) were analytical grade purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Methanol, hexane, acetic acid, and 2,4-dimethylpyridine (2,4-DMP) were purchased from Merck (Darmstadt, Germany). Deionised water was obtained from a MiliQ UV-Plus water purification system (Millipore Corp., Billerica, MA). Four isoforms of vitamin E homologue, α-tocopherol, α-tocotrienol, γ-tocotrienol, and δ-tocotrienol, were from Sigma-Aldrich, and two isoforms, β-tocopherol and γ-tocopherol, were from Eisai Food & Chemical Co. Ltd. (Japan). The standard compound 2AP was synthesized as outlined by Buttery, Juliano, and Ling (1983), with some modifications according to a previous study (Sriseadka, Wongpornchai, & Kitsawatpaiboon, 2006). Nylon/linear low-density polyethylene (nylon/LLDPE) pouches and aluminum pouches were purchased from Chiang Mai Plastic Co., Ltd. (Chiang Mai, Thailand).

2.3. Drying methods and storage conditions

The fresh paddy of LP rice was harvested and dehydrated using two different methods: sun drying and hot air drying (block-drying) at 40 °C until the moisture content was reduced to 14%. The times taken by these drying processes were 52 h for sun drying and 9 h for hot air drying. After drying, the rice samples were divided into two groups. One group was stored at room temperature of 30 °C and the other group was stored in a cool room at 22 °C. Both groups were stored for 10 months. For cool room storage, a number of industrial air conditioners were used and were set to maintain a temperature of 22 °C.

2.4. Packaging methods

Two weeks after harvesting and drying, the JHN rice was de-hulled and samples were sealed in four package types: nylon/LLDPE pouches; nylon/LLDPE pouches filled with N_2; nylon/LLDPE pouches kept under vacuum at 0.8 bar; and aluminum pouches kept under vacuum at 0.8 bar. These black rice samples were then kept at room temperature for 6 months.

2.5. Analysis of anthocyanins, γ-oryzanols, and vitamin E

2.5.1. Extraction

Rice samples were ground with a blender (Y46; Moulinex, Paris, France) before phytochemicals were extracted. One gram of each of the ground rice seed samples was used to determine anthocyanins and γ-oryzanols and two grams of the ground samples were used to determine vitamin E. Acetic acid (0.5%) in methanol (10 mL), methanol (10 mL), and hexane (15 mL) were used for extracting anthocyanins (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009), γ-oryzanols, and vitamin E (Chen & Bergman, 2005), respectively. For the analysis of anthocyanins, 1 mL of internal standard naringin (1 mg/mL) was added. After shaking for 20 min, this mixture was filtered and the solution was evaporated under reduced pressure at 40 °C until its volume was 2 mL of anthocyanin extract and 1 mL of γ-oryzanol and vitamin E extracts. The anthocyanin extract of LP rice samples was then diluted 10-fold with methanol. All the extracts were filtered through 0.45 μm pore nylon syringe filter prior to analysis by high-performance liquid chromatography (HPLC).

2.5.2. Determination of anthocyanins by HPLC-PDA and LC-MS

Quantitative analyses of anthocyanins in the black rice extracts were done using a Waters liquid chromatograph system (Waters Corp., Milford, MA) equipped with a 2695 separation module and a 2996 photo-diode array (PDA) detector. Separation was done on a 2.1 × 150 mm Halo C18 column (Advanced Materials Technology, Wilmington, DE) with 2.7 μm particle size. The system's mobile phase was methanol, solvent A, and acetic acid in water (0.5:99.5), solvent **B**, at a flow rate of 0.1 mL/min. The injection volume was 5 μL and the detection was at 520 nm. The elution started