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A study on the color deepening in red rice during storage

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ABSTRACT

To elucidate the mechanism of the color deepening phenomenon involving aged red rice samples, the time-dependent changes in the color and chemical composition of four different samples (freshly harvested, as well as those stored for 1, 2, and 5 years) were studied. A detailed study of the color change was carried out by obtaining the reflectance spectra of the samples. To investigate the underlying chemical changes, each sample was extracted with an appropriate solvent and analyzed by high-performance liquid chromatography, Folin–Ciocalteu assay, phloroglucinolysis, and mass spectrometry. A similar color change was induced via the photoirradiation of a fresh red rice sample. It is suggested that the chemical modifications of proanthocyanidins are responsible for the color change observed in red rice.

1. Introduction

Various types of rice (*Oryza sativa*) are widely cultivated around the world, and those with pigments are commonly known as the colored rice varieties. Almost all pigments are contained within the bran layers and colored rice can be classified as red and black rice based on the difference in color. The main pigment of black rice is anthocyanin and this bioactive constituent exhibits beneficial biological properties including anti-oxidative, anti-inflammatory, and anti-carcinogenic activities (Chen, Choi, Kozukue, Kim, & Friedman, 2012; Itani, Tatemoto, Okamoto, Fujii, & Muto, 2002; Kim et al., 2008; Min, McClung, & Chen, 2011). On the other hand, the main pigments of red rice are proanthocyanidins. They are composed of flavan-3-ol units, with an average degree of polymerization (DP) of 2–14, and these bioactive constituents exhibit beneficial biological properties, including strong anti-oxidative activity (Chen et al., 2012; Itani et al., 2002; Min et al., 2011; Oki et al., 2002). The color of red rice intensifies during the ripening process, and its intensity depends on the crop's growing conditions. Furthermore, it changes to a darker reddish-brown hue during the post-harvest storage period. It is unlikely that any new pigment is produced by the red rice during the storage period, and it is postulated that the color deepening phenomenon is a result of changes occurring in preexisting chemical species. In order to elucidate the mechanism of color deepening in red rice, the time-dependent changes in the color and chemical composition of different rice samples were investigated.

2. Materials and methods

2.1. Materials

Four batches of red rice from different picking seasons (in 2008, 2011, 2012, and 2013) were used for this study. The sample harvested in 2013 was defined as "year 0." Red rice samples were obtained from the Gifu Field Science Center at Gifu University. All reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Reflectance spectra

Reflectance spectra were recorded on a Perkin Elmer Lambda 950 UV/VIS/NIR spectrophotometer (Kanagawa, Japan), equipped with an integrating sphere. The spectra of each sample, recorded against a standard white board, were taken in the range between 200 and 2500 nm, with a spectral resolution of 1 nm. Spectral data were subsequently processed using Microsoft Excel 2013. The absorbance values were calculated from the relative reflectance (R%) as $\mathrm{Abs} = \log(100/R)$.

2.3. Rice bran extracts

The rice grains were soaked in deionized water at a 1:10 bran-to-solvent ratio under stirring for 5 h at 35 °C. The aqueous extract was filtered and the filtrate was loaded into a column packed with Diaion HP20SS particles (Mitsubishi Chemical Corporation, Tokyo, Japan), before being eluted with MeOH. The column eluates were concentrated under reduced pressure and weighed. After the initial

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aqueous extraction, the rice bran was extracted with 2.5% trifluoroacetic acid (TFA) in MeOH (at a 1:10 bran-to-solvent ratio) at rt (left overnight), and a further 7 h with dimethylsulfoxide (DMSO). Saccharides and amino acids were removed from the MeOH and DMSO extracts, respectively, using the Diaion HP20SS column. The column eluates were concentrated under reduced pressure and weighed.

2.4. High-performance liquid chromatography analysis

High-performance liquid chromatography (HPLC) analyses were carried out with a JASCO PU-2089 intelligent pump, equipped with a JASCO MD-2010 PDA detector and JASCO CO-2065 column oven (Tokyo, Japan). The individual extracts were dissolved in MeOH and analyzed on a C18 analytical column (NB-ODS-9, 4.6 mm id × 250 mm; Nagara Science Co., Ltd., Gifu, Japan). The mobile phase consisted of 1% AcOH:45% MeOH in H_2O. In addition, the extracts were also analyzed on a hydrophobic interaction liquid chromatography (HILIC) column (Inertsil Amide, 4.6 mm id × 250 mm; GL Sciences Inc., Tokyo, Japan). With this column, the mobile phase was switched to 1% AcOH:85% MeCN in H_2O (for the aqueous extract) and 1% AcOH:90% MeCN in H_2O (for the MeOH extract). The analyses were carried out at 35 °C, and the flow rate was set at 1.0 mL/min.

2.5. Folin–Ciocalteu analysis

The total phenolic content was determined using the Folin–Ciocalteu method (Turkmen, Sari, & Velioglu, 2006). A standard curve of gallic acid (with concentrations ranging from 0.0078 to 0.063 mg/mL) was prepared. The results were determined from a regression equation of the standard curve ($y = 11.314x + 0.0202$), and expressed as milligram gallic acid equivalents per gram of sample. The rice bran extract was diluted with deionized water to give concentrations ranging from 0.0078 to 0.063 mg/mL. In this assay, 0.5 mL of the diluted solution was first mixed with 0.2 mL of Folin–Ciocalteu's reagent. Next, 0.5 mL of saturated aqueous sodium bicarbonate solution was immediately added to the mixture, shaken thoroughly, and diluted to 5.0 mL by the addition of water (4.3 mL). The mixture was allowed to stand for 2 h and its absorbance measured at 725 nm using a UVmini-1240 spectrophotometer (Shimadzu, Tokyo, Japan).

2.6. Phloroglucinolysis

Phloroglucinol (200 mg) and ascorbic acid (40 mg) were dissolved in MeOH (2.0 mL) containing 0.1 M HCl (4.0 mL). The aqueous extracts (10 mg each) were added to this solution separately and left to react at 50 °C for 20 min, followed by the addition of the same volume of 40 mM aq. NaOAc to stop the reaction. The product was analyzed using the HPLC-photo diode array (PDA) technique, with a C18 analytical column (NB-ODS-9, 4.6 mm id × 250 mm). The mobile phase consisted of 1% AcOH:10% MeCN in H_2O. The column temperature and flow rate were set at 35 °C and 1.0 mL/min, respectively. The reaction products were purified using preparative HPLC and analyzed via ^1H NMR spectroscopy. The NMR spectra were recorded using tetramethylsilane (TMS) as an internal standard on a JEOL ECA-600 instrument (Tokyo, Japan). The chemical structures were determined using NMR spectroscopy and confirmed by literature sources (Kennedy & Jones, 2001; Kohler & Winterhalter, 2005).

2.7. Mass spectrometry analysis

LC-electron spray ionization (ESI)-mass spectrometry (MS) was performed using a Waters Xevo G2 QTof (Waters, USA) mass spectrometer, equipped with a C18 analytical column (ACQUITY UPLC BEH C_{18} 2.1 mm id × 100 mm; Waters, USA). The mobile phase consisted of 1% AcOH:45% MeOH in H_2O. The analyses were carried out at 35 °C, and the flow rate was set at 0.4 mL/min.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI- TOF) mass spectra were collected on a Shimadzu AXIMA Resonance mass spectrometer (Shimadzu, Japan), equipped with delayed extraction and a N_2 laser set at 337 nm. The spectra were calibrated with angiotensin II (M_W 1046.6 Da) and adrenocorticotropic hormones clip 18–39 (ACTH clip 18–39; M_W 1296 Da) as external standards. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix and dissolved in 30% acetonitrile in H_2O (10 mg/mL). Meanwhile, the samples were dissolved in methanol (10 mg/mL). The matrix and sample solutions were mixed (1:1, v/v) and the mixture was deposited onto the MALDI target, and left to crystallize at room temperature (Howell et al., 2005; Monagasa, Quintanilla-López, Gómez-Cordovésa, Bartoloméa, & Lebrón-Aguilarc, 2010; Ohnishi-Kameyama, Yanagida, Kanda, & Nagata, 1997).

2.8. Photoirradiation study

"Year 0" red rice sample was exposed to LED light (wavelength range: 450–495 nm; model 7-PR30-01, Edison Opto Corporation, Taiwan). The red rice samples were spread evenly on a dish and the LED light irradiated from above. The distance between the LED light source and the rice sample was fixed at 15 cm. The total irradiation time lasted anywhere between 24 and 1514 h. Subsequently, the irradiation product was analyzed using reflectance spectroscopy, before being extracted and its sample collected for HPLC-PDA analysis.

3. Results and discussion

3.1. Ultraviolet/visible reflectance measurements

The color of the red rice sample was observed at different storage durations, i.e., just after harvest ("year 0") and after 1, 2, and 5 years of storage (the aging process). "Year 0" (freshly harvested) sample appeared as light orange whereas the other samples were dark red in color. This observation suggested that the change in sample color occurred rapidly during the first year of storage, but became increasingly less noticeable from the second year onward. For a detailed observation of the color change over a total period of 5 years, the samples' reflectance spectra were obtained (Fig. 1). Although there was no significant change in the maximum absorption wavelength, the spectral absorption between 400 and 600 nm increased with the duration of storage. The absorption spectrum of the "year 0" sample was noticeably different from the other aged samples. This confirmed our visual observations and indicated that the change in sample color is likely a result of altered chemical structures after one year of storage post-harvesting.

3.2. Solvent extraction

Various solvents have been used for the extraction of chemical components from plants, and the extraction yield fluctuates depending on the type of solvent used. In this study, each red rice sample was extracted using water, MeOH, and DMSO (in the listed order), and their respective extracted contents were measured accordingly (Fig. 2A). In general, the total extracted contents decreased with a prolonged storage period. Evidently, the MeOH extract constituted approximately 50% of the total extracted content of all four samples. The maximum aqueous extract content was obtained from the "year 0" sample, with minimal content