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Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking

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ABSTRACT

As hydrophilic pigments, anthocyanins reduce the incidence of cancer and cardiovascular diseases. In this study, the anthocyanin content and composition following steaming and baking of the roots of the Korean purple-fleshed sweet potato variety "Shinzami" were evaluated using liquid chromatography with diode array detection and electrospray ionisation/mass spectrometry (LC-DAD-ESI/MS). Anthocyanins of Shinzami were composed of mono- or di-acylated forms of *p*-hydroxybenzoic acid, caffeic acid and ferulic acid with the basic structure of cyanidin 3-sophoroside-5-glucoside or peonidin 3-sophoroside-5-glucoside. A total of 15 individual anthocyanins were isolated and confirmed, one of which was presumed to be a newly identified compound, peonidin 3-feruloyl-*p*-hydroxybenzoyl sophoroside-5-glucoside. Additionally, the amounts of di-acylated cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside and peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside were the highest (137.0 and 565.9 mg/100 g DW, respectively) among cyanidin and peonidin compounds. After steaming, the total anthocyanin content was reduced by nearly a half, while roasting only slightly reduced the total anthocyanin content.

1. Introduction

Hydrophilic anthocyanin pigments are a class of flavonoid compounds responsible for the blue, purple and red colours of most plants. To date, 23 basic anthocyanidins (aglycones) have been identified, and the six most common ones in plants are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. In nature, thousands of anthocyanin compounds have been identified, whose structures vary with respect to the type and number of sugars, organic acids and phenolic acids present (Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009; Escribano-Bailon, Santos-Buelga, & Rivas-Gonzalo, 2004; Kahkonen & Heinonen, 2003; Kong, Chia, Goh, Chia, & Brouillard, 2003). Anthocyanins exert a strong anticancer effect through their antioxidant and anti-inflammatory activities, as well as through their abilities to induce cell proliferation inhibition, cell cycle arrest and apoptosis in specific cancer cells (Kong et al., 2003; Wang & Stoner, 2008).

Sweet potato is a highly nutritious vegetable that is rich in vitamins (B_1, B_2, C and E), minerals (Ca, Mg, K and Zn), dietary fibre, and dietary carbohydrates. In addition to the listed nutritive components, purple-fleshed sweet potatoes contain large quantities of anthocyanins, which have excellent biological properties and potential value as natural colours for foods (Suda et al., 2003). The anthocyanins in purple-fleshed sweet potato primarily occur as acylated compounds. Currently, the biological activity of anthocyanins from the sweet potato cultivar Ayamurasaki, which was developed in Japan in 1995, is being evaluated.

Two mono-acylated forms, cyanidin 3-caffeoyl sophoroside-5-glucoside and peonidin 3-caffeoyl sophoroside-5-glucoside, were first isolated and identified using FAB-MS and NMR from purple-fleshed sweet potato (*Ipomoea batatas* L. Cv. Yamagawamurasaki) (Goda et al., 1997; Odake, Terahara, Saito, Toki, & Honda, 1992). Six anthocyanins di-acylated with *p*-hydroxybenzoic acid, caffeic acid and ferulic acid were subsequently identified (Terahara et al., 1999), followed by the identification of two non-acylated forms, cyanidin 3-sophoroside-5-glucoside and peonidin 3-sophoroside-5glucoside, which brought the total anthocyanin number to ten in this plant tuber (Saigusa, Terahara, & Ohba, 2005). Recently, a reference-based interpretation of MS fragment spectra found 17 non-, mono- or di-acylated individual anthocyanins in three sweet potato cultivars (Stokes Purple, NC 415 and Okinawa) using LC-DAD/ESI-MS/MS (Truong et al., 2010). Further, from callus culture extracts

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prepared from the storage roots of purple-fleshed sweet potato (*Ipomoea batatas* L. Cv. Ayamurasaki), 22 anthocyanins were isolated and identified using LC-ESI-MS/MS, which added anthocyanins acylated with *p*-hydroxybenzoic acid, caffeic acid, ferulic acid and *p*-coumaric acid, based on cyanidin, peonidin and pelargonidin (Konczak-Islam, Okuno, Yoshimoto, & Yamakawa, 2003; Terahara et al., 2000; Tian et al., 2005a,b). In the Ayamurasaki cultivar, peonidins and cyanidins accounted for 74% and 19% of the acylated anthocyanins, respectively (Tsukui et al., 1999). The total anthocyanin content of the cultivar was approximately 0.6 mg/g fresh weight, with the primary component being peonidin 3-caffeoyl sophoroside-5-glucoside (Furuta, Suda, Nishiba, & Yamakawa, 1998).

Acylated anthocyanins from purple-fleshed sweet potato have been reported to show high pH stability, thermostability, antioxidant activity and antimutagenicity (Kano, Takayanagi, Harada, Makino, & Ishikawa, 2005; Terahara and Matsui, 2008; Yoshimoto, Okuno, Yamaguchi, & Yamakawa, 2001; Yoshimoto et al., 1999, 2001). An analysis of anthocyanins from blood plasma or urine in animal models (including humans) found that when the potato was eaten raw or consumed in beverage form, only 0.01–0.03% of the anthocyanins remained upon excretion. This indicates that the anthocyanins were not only selectively well absorbed but were also well maintained in their original form (Harada, Kano, Takayanagi, Yamakawa, & Ishikawa, 2004; Oki, Suda, Terahara, Sato, & Hatakeyama, 2006; Suda et al., 2002).

Shinzami, a Korean purple-fleshed sweet potato variety created by crossing Yamagawamurasaki with Shinmi, was propagated as a leading variety for pigment production in 2001 following a selection process. However, in Korea, there have been few qualitative and quantitative studies of anthocyanins in comparison to Japan. Therefore, an urgent need exists to find individual anthocyanins suitable as selection indicators at the plant breeding stage. For that purpose, anthocyanins should be profiled promptly and on a large scale by making active use of the libraries of MS and UV spectra and relative response factors (RRFs).

In general, anthocyanin analysis involves mass spectrometry methods, such as tandem MS (Giusti, Rodriguez-Saona, Griffin, & Wrolstad, 1999; Tian et al., 2005a,b). However, this study employs a precise MS fragment interpretation of high molecular weight sweet potato anthocyanins despite the use of single MS. Additionally, this technique enables high peak separation and reproducibility with only a small amount of sample, as well as the implementation of prompt and large-scale screening for the evaluation of sweet potato germplasm by reducing the length of the analysis time. Previously, quantification had been limited to specific anthocyanins according to the availability of their standard samples. However, using this method, it was possible to quantify all of the individual anthocyanins separated by the use of an internal standard solution.

Consequently, this study attempted to identify the structures of individual anthocyanins in the Korean purple-fleshed sweet potato variety "Shinzami" using an LC-DAD-ESI/MS method and to assess how their contents changed according to the processing method employed.

2. Materials and methods

2.1. Materials

For this study, the Korean purple-fleshed sweet potato variety "Shinzami", distributed in 2010 from the Bioenergy Crop Research Center, was divided into three different processed types: raw, steamed (for 10 min at 121 °C) and baked (for 40–50 min at 200 °C). The samples were freeze dried and finely ground with a sample mill for use as analytical samples.

2.2. Instrumentation and reagents

The instruments used during the pretreatment process included a refrigerated multi-purpose centrifuge (Hanil Science Industrial Co. Ltd., Korea), and a digital precise shaking water bath (Daihan Scientific Co. Ltd., Korea). Cyanidin 3,5-diglucoside (Extrasynthèse, France) was used as an internal standard solution. The HPLC reagents were water, methanol and acetonitrile from J. T. Baker (Phillipsburg, NJ) and formic acid from Sigma (St. Louis, MO).

2.3. Extraction

A 1-g powder sample in a conical tube (50 mL) was centrifuged (3000 rpm, 10 min, 4 °C) following extraction with 20 mL of 5% formic acid in H_2O for 24 h at 40 °C in a shaking water bath. One millilitre of the supernatant solution was collected from the centrifuged sample. A Sep-Pak C_{18} cartridge was flushed with 2 mL of MeOH, followed by the addition of 2 mL of H_2O for activation. After loading 1 mL of supernatant (anthocyanin extract) and 1 mL of internal standard solution (cyanidin 3,5-diglucoside, 100 ppm), the cartridge was washed with 2 mL of H_2O and eluted with 1 mL of MeOH. The anthocyanin filtrate was eluted and concentrated using N_2 gas, and then re-dissolved in 1 mL of 5% formic acid in H_2O prior to analysis with LC-DAD-ESI/MS.

2.4. Quantitative and qualitative analysis of anthocyanins by LC-DAD-ESI/MS

The anthocyanins of purple-fleshed sweet potato were identified and quantified using a Micromass ZQ MS (Waters Co., Milford, MA) and an Alliance e2695 HPLC system (Waters Co.) equipped with a 2998 photodiode array detector (PDA), in addition to a Synergi Polar-RP 80A reversed-phase column (4.6 × 250 nm I.D., 4 μm; Phenomenex, Torrance, CA). The analysis was conducted at a flow rate of 1 mL/min at a detection wavelength of 250–600 nm (a representative wavelength of 525 nm) and the oven temperature was 30 °C. The mobile phases used were 5% formic acid in water (phase **A**) and 5% formic acid in water/acetonitrile (1:1, v/v) (phase **B**) (Oh et al., 2008). The pretreated sample was analysed by using the following gradient conditions; a gradient of 20–50% **B** over a 30 minute period; 50% **B** for five minutes; a gradient of 50% to 20% **B** for five minutes; and then a final wash with 20% **B** for ten minutes. MS analysis was run in positive ionisation mode using an electrospray ionisation (ESI) source. The MS parameters were each set to a cone voltage of 30 V, source temperature of 120 °C, desolvation temperature of 500 °C, and a desolvation N_2 gas flow of 1020 L/h. The range of molecular weights was *m/z* 200–1200 in full scan mode.

2.5. LC–MS library for qualitative analysis of anthocyanins

Based on a variety of literature sources, an LC–MS library of 31 different anthocyanins in purple-fleshed sweet potato was created and used for the efficient determination of individual components (Harada et al., 2004; Tian et al., 2005a,b; Truong et al., 2010).

3. Results and discussion

Fig. 1 shows an HPLC chromatogram of anthocyanins from a water extract of Shinzami, a Korean-cultivated purple-fleshed sweet potato variety. A total of 15 different compounds, including one unknown, was isolated and identified on the PDA-detected UV and LC-ESI/MS spectra with reference to the LC–MS library of purple-fleshed sweet potato anthocyanins. When analysed in the positive ionisation mode by a single quadrupole MS system with an ESI