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An analytical pipeline to compare and characterise the anthocyanin antioxidant activities of purple sweet potato cultivars

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ABSTRACT

Purple sweet potato (*Ipomoea batatas* L.) is rich in anthocyanin pigments, which are valuable constituents of the human diet. Techniques to identify and quantify anthocyanins and their antioxidant potential are desirable for cultivar selection and breeding. In this study, we performed a quantitative and qualitative chemical analysis of 30 purple sweet potato (PSP) cultivars, using various assays to measure reducing power radical-scavenging activities, and linoleic acid autoxidation inhibition activity. Grey relational analysis (GRA) was applied to establish relationships between the antioxidant activities and the chemical fingerprints, in order to identify key bioactive compounds. The results indicated that four peonidin-based anthocyanins and three cyanidin-based anthocyanins make significant contributions to antioxidant activity. We conclude that the analytical pipeline described here represents an effective method to evaluate the antioxidant potential of, and the contributing compounds present in, PSP cultivars. This approach may be used to guide future breeding strategies.

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is an edible crop with high levels of vitamins (A, B_1, B_2, C, and E), minerals (Ca, Mg, K, and Zn), dietary fibre and carbohydrates (Kim et al., 2012; Scott, Best, Rosegrant, & Bokanga, 2000). Purple sweet potato (PSP) is a particularly nutritionally valuable variant of sweet potato since, in addition to the nutritive components mentioned above, it is also rich in anthocyanins; flavonoid pigments that contribute a red, blue or purple colour to the organs of vascular plants (Kim et al., 2012).

The anthocyanins in PSP (PSPAs) are mostly 3,5-diglucoside derivatives of cyanidin or peonidin, with caffeoyl, feruloyl, and *p*-hydroxybenzoyl residues as additional variants (Kim et al., 2012). PSPAs exhibit diverse biological activities, including antioxidant (Wu, Tsai, Hwang, & Chiu, 2012; Zhang et al., 2013), anticancer (Lim et al., 2013; Ye, Meng, Yan, & Wang, 2010), _ENREF_7antidiabetic (Jang, Kim, Kim, Kim, & Lee, 2013; Zhao, Yan, Lu, & Zhang, 2013), anti-inflammatory (Wang et al., 2010; Zhang et al., 2009), and hepatoprotective activities (Sun, Mu, Liu, Zhang, & Chen, 2014; Wang, Tong, et al., 2014; Wang, Li, et al., 2014). Moreover, PSPAs containing aromatic acylated glycosyl groups have been shown to have high pH and thermo-stability (Giusti & Wrolstad, 2003; Kim et al., 2012). Thus, PSP represents a valuable source of natural food colourants and functional food ingredients (Oki et al., 2002; Steed & Truong, 2008). There is growing interest in both the fresh market and processing industries in expanding use of PSP worldwide (Cevallos-Casals & Cisneros-Zevallos, 2004; Giusti & Wrolstad, 2003).

Traditional PSP breeding efforts have mainly focused on developing cultivars with high levels of anthocyanins, and such efforts have typically involved measuring antioxidant capacity and total anthocyanin content, while it is well known that different anthocyanin compounds exhibit different biological activities (Kähkönen & Heinonen, 2003; Nayak, Berrios, Powers, & Tang, 2011; Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002; Wu, Pittman, & Prior, 2006). Consequently, there is a need to determine whether a particular anthocyanin(s) is primarily responsible for the antioxidant activity.

In the present study, 30 PSP cultivars developed in Chongqing, PR China were selected for an assessment of antioxidant activity. Antioxidant activities were evaluated *in vitro* using a reducing power activity (RPA), DPPH radical (DPPH·) scavenging activity, hydroxyl radical (OH·) scavenging activity and linoleic acid autoxidation (LAAO) inhibition activity assays. Specific anthocyanins

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were identified by high-performance liquid chromatography diode array detector tandem mass spectrometry (HPLC–DAD–MS/MS). The relationship between antioxidant activity and the chromatographic information was established by a chemometric analysis method, grey relational analysis (GRA). After analysis of the GRA data, the key contributors of antioxidant activity could be predicted. This information has potential value for the screening of PSP cultivars with significant antioxidant activities for plant breeding, and for the development of PSP-based functional foods with improved health benefits.

2. Materials and methods

2.1. Plant material and chemicals

Thirty cultivars of PSP (Table 1) were obtained from the Chongqing Engineering Research Centre for Sweet Potato (Chongqing, China). The roots from these cultivars were harvested, cut, freeze dried, powdered in liquid nitrogen, and stored at −20 °C until use.

The analysis used HPLC-grade acetonitrile and trifluoroacetic acid (Fisher Scientific, Fair Lawn, NJ) and distilled deionised water (Purelab classic system, Elga, High Wycombe, UK). Ethanol, trichloroacetic acid, hydrogen peroxide (H_2O_2, 30%), 1,10-phenanthroline, and linoleic acid were analytical grade (Kelong Chemical Co., Ltd., Chengdu, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was provided by Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Macroporous resin AB-8 was obtained from The Chemical Plant of Nankai University (Tianjin, China).

2.2. Sample preparation

Extractions of PSPAs were performed as previously described (Li et al., 2013), with some modifications. Briefly, each sample of PSP powder (50.0 g) was centrifuged for 15 min (4000 rpm, 3500*g*, 4 °C) after extraction with 1.0 L 50% aqueous ethanol containing 0.05% hydrochloric acid for 24 h at 4 °C in the dark. The supernatant was concentrated by evaporation under vacuum at 40 °C and the concentrated extract was applied to a macroporous resin AB-8 column that had been pre-equilibrated with water containing 1% formic acid. The column was then equilibrated with three column volumes (3 CV) of the equilibrium liquid to remove sugars and proteins. PSPAs were then eluted with 3 CV 70% aqueous ethanol containing 1% formic acid; the eluent was concentrated under *vacuum* (40 °C) to remove the ethanol from the solution. The concentrate was partitioned with ethyl acetate (200 mL × 3) to remove other phenols (Oki et al., 2002). The aqueous fraction was then lyophilised (−40 °C) to obtain a dry powder, 10% of which was weighed and dissolved in 100 mL water prior to total anthocyanin content (TAC) analysis, while the remaining powder was stored at −20 °C for use in the other experiments.

Table 1
Summary of 30 tested PSP cultivars.

Sample No.	Cultivar name	Sample No.	Cultivar name
S1	6-6-12	S16	7-33-15
S2	6-6-16	S17	7-35-2
S3	6-8-37	S18	7-44-10
S4	6-12-2	S19	8-3-3
S5	Yuzishu No. 3[a]	S20	8-4-11
S6	Yuzishu No. 7[a]	S21	8-13-28
S7	6-13-32	S22	8-13-49
S8	Qianzishu No. 1[a]	S23	8-24-15
S9	6-21-27	S24	8-33-5
S10	6-24-50	S25	Rizishu-7[a]
S11	7-11-8	S26	Rizishu-13[a]
S12	7-12-21	S27	8-45-9
S13	7-28-2	S28	Wanzishu-56[a]
S14	7-28-10	S29	Yusuzi-43[a]
S15	7-33-2	S30	Yuzishu-263[a]

[a] Names followed by letters are approved purple sweet potato cultivars in China. Other materials were in attending regional test.

2.3. HPLC analysis and LC–MS identification

PSPA extracts were dissolved in water containing 0.05% hydrochloric acid and passed through a 0.22-μm syringe filter (Jinteng Experiment Equipment Co., Ltd, Tianjin, China) prior to HPLC analysis using a Shimadzu LC-20 HPLC system (Shimadzu Corp., Kyoto, Japan) equipped with an SPD-M20A diode array detector (DAD), two LC-20AD pumps, a CTO-20A column oven, a DGU-20A_{3R} degasser and an SIL-20A autosampler, all controlled with LCsolution (Version 1.24 SP1, Tokyo, Japan). The separations were carried out on a Kromasil C18 column (150 × 4.6 mm; Eka Chemicals AB, Bohus, Sweden) equipped with a Kromasil guard column kit. The injection volume was 10 μL and a binary gradient elution mixture, composed of water with trifluoroacetic acid (99.5/0.5, v/v) (**A**) and acetonitrile (**B**) was applied to the column, as follows: 0–8 min, 12–16% **B**; 8–12 min, 16–17% **B**; 12–16 min, 17–19% **B**; 16–20 min, 19% **B**; 20–22 min, 19–20% **B**; and 22–30 min, 20% **B**. The mobile phase flow rate was 1.0 mL/min, the temperature of the column oven was set to 30 °C and the DAD was set to 530 nm for acquiring chromatograms recorded from 200 to 800 nm.

Liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS) analysis was performed on a Shimadzu UFLC XR system connected to a Shimadzu LC MS-8030 triple quadrupole mass spectrometer. The HPLC column, column oven temperature and conditions of the mobile phase gradient for this analysis were as described above for the HPLC analysis. Mass spectra with an *m*/*z* range of 500–1500 were obtained in positive-ion mode. The conditions of the ESI source were optimised as follows: nitrogen was used as the nebulising gas and drying gas, with flow rates of 3.0 and 15.0 L/min, respectively. The temperatures of the heat block and desolvation line were set at 400 and 250 °C, respectively. LC–MS/MS analysis was performed with the same instrument and conditions as described above, with collision energies from 10 to 25 eV.

2.4. Determination of total anthocyanin content (TAC)

The TAC was determined using a spectrophotometric pH differential method (Giusti & Wrolstad, 2001) with some modifications. Two dilutions were performed for each sample, one with hydrochloric acid–potassium chloride buffer (0.025 M, pH 1.0) and the other with sodium acetate buffer (0.4 M, pH 4.5). Difference in absorbance of the two dilutions at different pH values is caused by a structural transformation of the constituent anthocyanins from the coloured oxonium at pH 1.0 to the colourless hemiketal at pH 4.5 (Giusti & Wrolstad, 2001). Samples were diluted to allow the absorbance readings to be in the linear range (<1.2) of a UV-1750 UV–Vis spectrophotometer (Shimadzu Corp., Suzhou, China). Samples were equilibrated for 15 min before the absorbance was recorded at 530 and 700 nm. Water was used as the blank. The absorbance was calculated as follows:

$$A = (A_{530} - A_{700})_{pH1.0} - (A_{530} - A_{700})_{pH4.5}$$

The TAC was calculated and expressed as cyanidin 3-glucoside equivalents using the following formula:

$$\text{Anthocyanin content (mg/L)} = A \times MW \times DF \times 1000/(\varepsilon \times L)$$

where *MW* was the molecular weight of anthocyanin (449.2 g/M), *DF* was the dilution factor (quotient of the final volume and the