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Fractionation, enzyme inhibitory and cellular antioxidant activity of bioactives from purple sweet potato (*Ipomoea batatas*)

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ABSTRACT

Sweet potato (*Ipomoea batatas* L.) is mainly cultivated in Asia. The deep purple color of purple sweet potato (PSP) is due to the high content of acylated anthocyanins. In the present study, PSP-derived polyphenols were identified using HPLC-PDA and HPLC-ESI-MS^n analyses. After concentration of the polyphenols from PSP, preparative separation into two fractions, designated anthocyanins (AF) and copigments (CF), was carried out using adsorptive membrane chromatography. In enzyme inhibitory assays, all PSP samples inhibited the enzymes α-amylase, α-glucosidase and xanthine oxidase. Additionally, the cell signaling cellular antioxidant properties of the PSP extracts were investigated in cultured cells. PSP induced the transcription factor Nrf2, which regulates the expression of genes encoding heme oxygenase 1 (*Hmox1*), glutamate-cysteine ligase catalytic subunit (*Gclc*) and paraoxonase 1 (PON1). Furthermore, PSP enhanced cellular glutathione concentrations and decreased lipid peroxidation in cultured hepatocytes. Overall, these results suggest that PSP extracts exhibit enzyme inhibitory and cellular antioxidant properties, especially PSP CF.

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is a root-derived food that belongs to the family Convolvulaceae and originated in Latin America. In the 16th century, sweet potato was introduced to Asia, Africa and Europe (Huamán, 1992). According to the Food and Agriculture Organization (FAO), sweet potato is a major world food crop, after sugar cane, rice, wheat, potatoes, maize, and cassava. In 2014, the world production of sweet potato was >100 million tons, and China is the largest sweet potato producer in the world (FAOSTAT, 2014). Sweet potato varieties differ in skin and flesh colors, e.g., white, yellow, orange, pink, purple and deep purple (Bradshaw, 2010; Teow et al., 2007).

Purple sweet potatoes (PSP) exhibit a deep purple color as a result of their relatively high content of acylated anthocyanins, with mainly cyanidin or peonidin as the aglycone (Konczak-Islam, Yoshimoto, Hou, Terahara, & Yamakawa, 2003; Zhang et al., 2015). It is suggested that anthocyanins, as natural pigments, may provide beneficial health effects. In several studies, antioxidant (Hu et al., 2016), anti-inflammatory (Sugata, Lin, & Shih, 2015), anticarcinogenic (Sugata et al., 2015), chemopreventive (Konczak-Islam et al., 2003) and antihyperglycemic (Matsui et al., 2002; Nagamine et al., 2014) properties of PSP and its constituents have been reported. Sweet potatoes are important staples of the traditional Okinawa (the southernmost prefecture of Japan) diet and are associated with longevity and low incidence of chronic degenerative diseases (Willcox & Willcox, 2014).

To date, anthocyanins are mainly separated from PSP by solvent extraction (e.g., alcohol with acid) (Hu et al., 2016; Zhang et al., 2015) and column chromatography (Matsui et al., 2002; Zhang et al., 2015). To the best of our knowledge, fractionation of other PSP-derived phenolic compounds has not yet been reported. Recently, a novel membrane chromatographic method was developed to fractionate polyphenols from bilberry (*Vaccinium myrtillus*) into an anthocyanin- and copigment-rich (i.e. colorless phenolic compounds) fraction (Juadjur & Winterhalter, 2012).

In the present study, polyphenols from PSP were characterized by HPLC-PDA (high-performance liquid chromatography-photodiode array detection) and HPLC-ESI-MS^n (high-performance liquid chromatography-electrospray ionization-multiple mass spectrometry) analyses. Fractionation of an anthocyanin-rich sweet potato XAD-7 extract into an anthocyanin- (AF) and copigment-rich fraction (CF) was carried out for the first time using adsorptive membrane chromatography, as described by Juadjur and Winterhalter (2012). Furthermore, the bioactivity of these PSP extracts in terms of their potential enzyme

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(e.g., α-amylase, α-glucosidase and xanthine oxidase) inhibitory activity was determined. In addition, the cell-signaling cellular antioxidant properties of purple sweet potato extracts were analyzed in cultured cells. The activity of the transcription factor Nrf2, a master regulator of antioxidant and stress response pathways, was determined using a reporter gene assay, and its antioxidant target genes such as heme oxygenase 1 (*Hmox1*), glutamate-cysteine ligase catalytic subunit (*Gclc*), glutathione synthetase (*Gss*) and glutathione peroxidase 4 (*Gpx4*) were identified using quantitative real-time PCR. Cellular glutathione was analyzed using a photometric method. The induction of the anti-atherogenic hepatic enzyme paraoxonase 1 (PON1) was determined using a reporter gene assay. Moreover, the BODIPY assay was applied to determine cellular lipid peroxidation in response to PSP treatment. Overall, comprehensive information about the enzyme inhibition and cellular antioxidant properties of PSP and its anthocyanins and phenolic acids is provided in this manuscript.

2. Materials and methods

2.1. Purple sweet potatoes (PSP)

Chinese sweet potatoes of the Purple Sweet variety were transported from Hefei (China) to Braunschweig (Germany) via air shipment within a week in April 2012. Samples exhibited a purple flesh and cortex, and a deep purple skin and cambium ring. To maintain product quality, sweet potatoes were stored dry and protected from light. The phenolic compounds from PSP were extracted one week after shipment. The obtained extracts were freeze-dried and stored at −20 °C until further analyses.

2.2. Chemicals

Authentic standards of cyanidin-3-*O*-glucoside and chlorogenic acid (5-CQA), Triton® X-100, DL-sulforaphane (⩾90%, HPLC), curcumin, the matrix substance for MALDI-MS (⩾99.5%, HPLC), acarbose (⩾95%) and cumene hydroperoxide, (technical grade, 80%) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol (HPLC grade), acetonitrile (HPLC grade) and formic acid (LC–MS grade) were obtained from Fisher Scientific (Loughborough, UK). Sodium hydroxide (⩾99%), sodium chloride (⩾99%), formic acid, p.a., dimethyl sulfoxide (DMSO; ROTIDRY® ⩾ 99.5%), resveratrol (⩾98%) and hemin chloride (⩾98%, crystalline) were provided by Carl Roth (Karlsruhe, Germany). Acetonitrile (LC–MS grade) was obtained from Honeywell Specialty (Seelze, Germany), and acetic acid, p.a. and hydrochloric acid (37%) were from Riedel-de-Haën (Seelze, Germany). Doubly deionized water was prepared using a Nanopure® resin (Nanopure; Barnstead, Thermo, Waltham, MA). Ethanol was distilled (industrial quality). C11-BODIPY (581/591) was purchased from Life Technologies (Darmstadt, Germany). Dulbecco's phosphate-buffered saline (DPBS) without Ca and Mg was obtained from PAN-Biotech (Aidenbach, Germany).

2.3. Extraction of anthocyanins

Approximately 7 kg of PSP were used for the extraction of anthocyanins. PSP were washed with water, dried and cut into small pieces (cube size of 1 cm^3) that included the peels due to their deep purple color. PSP were blanched at 100 °C for 3 min with Nanopure® water. A solution of water/HCl (19:1, v/v) was added, cooled at 0 °C for 3 h and stored at room temperature for 8 h. The suspension was filtered to remove solids. This crude extract was applied onto an Amberlite® XAD-7 HP column (Sigma, Steinheim, Germany), in order to eliminate sugars, proteins and organic acids. The column was washed with Nanopure® water, and anthocyanins were eluted with a solution of methanol/acetic acid (19:1, v/v). The eluate was concentrated *in vacuo*, dissolved in water and freeze-dried to obtain the PSP XAD-7 extract.

2.4. Adsorptive membrane chromatography (Juadjur & Winterhalter, 2012)

The separation of anthocyanins and copigments from the PSP XAD-7 extract was achieved using the membrane adsorber Sartobind S IEX (150 mL), from Sartorius Stedim Biotech (Goettingen, Germany). This membrane adsorber is a strong acidic cation exchanger with sulfonic acid groups ($R–SO_3^-$) on its surface. To isolate anthocyanins, conversion into positively charged flavylium cations by acidification is indispensable; cations are retarded at the membrane adsorber surface and thus selectively separated from other polyphenols.

Ten grams of PSP XAD-7 extract were dissolved in 1 L methanol/acetic acid (19:1, v/v). To protect the membrane adsorber, filtration of the extract solution was performed using filter paper (MN 615 ¼; Macherey-Nagel, Düren, Germany). A Sartopore 2300 filter capsule was connected between the adsorber and the pumping system. Regeneration and equilibration of the membrane absorber was carried out with 2.5 L of 1 N NaOH, 2.5 L of 0.01 N HCl and 1 L of methanol/acetic acid (19:1, v/v) using a Tandem 1082 peristaltic pump from Sartorius with a flow rate of 100 mL/min. After these steps, the extract solution was loaded. To remove copigments, the membrane adsorber was washed with 1 L of methanol/acetic acid (19:1, v/v) and copigments were collected. The retarded anthocyanins were eluted with 1 L of a 1:1 (v/v) mixture of aqueous 1 M NaCl and methanol. In addition, the anthocyanin fraction was acidified with acetic acid to a final concentration of 1% for the stabilization of anthocyanins.

During the loading, washing and elution steps (200 mL each), a 2-mL sample of the solution was collected, dried under nitrogen and analyzed by HPLC-PDA. The solvents of the copigment (CF)- and anthocyanin (AF)-fraction were removed *in vacuo*, and both fractions were freeze-dried.

AF contained sodium chloride. For this reason, a desalination step was necessary for the anthocyanin fraction using Amberlite® XAD-7 HP column chromatography. AF was dissolved in Nanopure® water/acetic acid (99.5:0.5, v/v) and applied onto the column. The column was washed with 3 L Nanopure® water/acetic acid (99.5:0.5, v/v) to eliminate salts. Anthocyanins were eluted with methanol/acetic acid (19:1; v/v), concentrated *in vacuo*, dissolved in Nanopure® water and freeze-dried.

2.5. High-performance liquid chromatography-photodiode array detection (HPLC-PDA)

The HPLC system consisted of a Jasco (Groβ-Umstadt, Germany) LG-980-02 ternary gradient unit with a DG-980-50 3 line degasser, an MD-910 multi-wavelength detector (wavelength range between 220 and 650 nm), a PU-980 Intelligent HPLC pump, an AS-950 Intelligent autosampler, and Borwin PDA chromatography software. The HPLC-PDA separations were performed on an RP-18 Luna 5μ C-18(2), 100 Å, 250 × 4.6 mm column (Phenomenex, Aschaffenburg, Germany) with a guard cartridge system of the same material. A flow rate of 0.5 mL/min was used. The injection volume was 20 μL. Two solvent systems were used. Solvent system A was a mixture of Nanopure® water/acetonitrile/formic acid (87:3:10, v/v/v), and solvent system B was Nanopure® water/acetonitrile/formic acid (40:50:10, v/v/v). The gradient was as follows: 0 min, 2% B; 20 min, 14% B; 25 min, 14% B; 40 min, 18% B; 45 min, 18% B; 70 min, 90% B; and then back to the initial conditions for 10 min. A hold time of 10 min was used before the next injection. The quantification of anthocyanins was performed with an