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Pre-heating and polyphenol oxidase inhibition impact on extraction of purple sweet potato anthocyanins

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ABSTRACT

Purple sweet potatoes (PSP) have been used as a natural food colorant with high acylated anthocyanins concentrations. Commercially extracting pigments from PSP can be challenging due to firm texture and high polyphenol oxidase (PPO) content. These studies evaluated hot water immersions (30, 50, 70, and 90 °C for 10 min) as pre-heating treatments and addition of PPO inhibitors (citric acid, oxalic acid, and sodium borate) to aqueous extraction solutions to aid pigment recovery. Predominant PSP anthocyanins included acylated cyanidin or peonidin derivatives. Non-pigmented cinnamates acted as oxidase substrates and induced co-oxidation reactions with anthocyanins. Pre-heating PSP significantly increased polyphenolic yields in a temperature-dependent manner, consistent with tissue softening and PPO inactivation. The use of solvent modifiers in the extraction solution associated with heat helped minimize enzyme action and increased polyphenolic recovery. Minimizing the impact of PPO with heat was critical to the extraction and recovery of PSP anthocyanins, suitable for food use.

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1. Introduction

Anthocyanins are pigments widely distributed in fruits, cereals, vegetables and are responsible for the intense red, blue and purple colors found in nature. Concentrated anthocyanin isolates from fruits and vegetables have gained popularity as natural color alternatives to synthetic red dyes and even other natural sources such as insect-derived carminic acid. In addition to color contributions, anthocyanins have reported health benefits such as reduction of reactive oxygen species, prevention of cardiovascular diseases, anti-inflammatory and anticarcinogenic activity, as reviewed by others (He & Giusti, 2010).

Purple sweet potatoes (PSP; *Ipomoea batatas*) with increased concentration of anthocyanins were originally cultivated in Japan, Korea, and New Zealand (Steed & Truong, 2008) but are currently grown, processed, and concentrated in the United States and other parts of the world with the intent to meet the growing market for natural colors. PSP anthocyanins are an attractive colorant for the food industry due to their high concentrations of stable, acylated anthocyanins that can provide colors ranging from pink to deep red depending on their concentration and solution acidity. Aromatic or aliphatic organic acids esterified to anthocyanin glycosides are more likely to possess greater initial color and are often more stable when exposed to conditions of food processing such as light, heat, and oxidative conditions (Giusti & Wrolstad, 2003). PSP possess anthocyanins that are predominantly acylated glucosides of cyanidin or peonidin although other anthocyanin glycosides have been reported in low concentrations (Truong et al., 2009). On previous studies, 13 of 15 anthocyanins in PSP were acylated with ferulic acid, diferulic acid, caffeic acid, or hydroxybenzoic acid moieties (Montilla et al., 2010), whereas acylation with *p*-coumaric acid in the cultivar 'Ayamurasaki' has also been identified (Konczak-Islam, Okuno, Yoshimoto, & Yamakawa, 2003; Tian, Konczak, & Schwartz, 2005). Besides anthocyanins, PSP extracts contain non-pigmented polyphenolics including chlorogenic, caffeic, and ferulic acids that not only serve as substrates for polyphenol oxidase (PPO; E.C. 1.14.18.1) but beneficially contribute to color as copigments and to antioxidant and biological activity of extracts as well (Huang, Chun-Der, Hsien-Jung, & Yaw-Huei, 2004; Yoshimoto et al., 1999).

Processing PSP for commercial anthocyanin recovery offers unique challenges, among which are the ability to rapidly inactivate PPO and inefficient extraction of pigments from the dense tissue structure. Although not considered substrates for PPO, anthocyanins will rapidly degrade with tissue disruption and posterior co-oxidation from hydrogen peroxide and *o*-quinone generation (Kader, Irmouli, Nicolas, & Metche, 2002). PPO catalyzes

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the O_2-dependent oxidation of phenolic acids to *o*-quinones that can be reactive to anthocyanins leading to loss of color and brown pigment formation (Thipyapong, Stout, & Attajarusit, 2007). However, some reports have indicated a direct action of PPO on anthocyanins in crushed fresh blueberries, and the addition of chlorogenic acid stimulated browning reactions that led to additional pigment destruction (Kader, Rovel, Girardin, & Metche, 1997). Additionally, PSP contain peroxidase (POD; EC 1.11.1.7) that will break down endogenous hydroperoxides, and those formed from PPO action may serve to exacerbate the degradation of anthocyanins at cut surfaces (Kader et al., 1997).

Thermal treatments with temperatures reaching up to 105 °C have been used to inactivate oxidase enzymes, considering optimal activity of enzymes such as polyphenol oxidase are near 40 °C (Yoruk & Marshall, 2003). Heat can also aid in extraction of pigments from fruits and vegetables including purple and red sweet potatoes (Suda et al., 2002; Cevallos-Casals & Cisneros-Zevallos, 2004). In addition to heat, the use of enzyme inhibiting agents added to extraction solvents may enhance extraction efficiency through increased ionic strength (Lu et al., 2010) and direct action as an oxidase enzyme inhibitor. Chelating acids such as citric or oxalic acid can bind to metal cofactors in enzymes (Yoruk & Marshall, 2003) and lower the pH below optimal activity levels. However, little knowledge exists to link the relationship between heat treatment and the role of chemical additives to inactivate oxidase enzymes to improve anthocyanin and phenolic acid extraction from PSP. Therefore, the aim of these studies was to evaluate the efficacy of pre-heating treatments and PPO-inhibiting chemical agents on the extractability of PSP polyphenolics and evaluate their stability for use as natural food colors.

2. Material and methods

2.1. Plant material and chemicals

Fresh purple sweet potatoes (PSP) from a proprietary development line (25.6 ± 0.6% dry matter) were obtained from Avoca Farms Inc., Merry Hill, North Carolina (USA) from the 2012 harvest, and 40 kg were transported the next day to the Department of Nutrition and Food Science at Texas A&M University. PSP were stored dry for 6 months at room temperature and shielded from direct light. Standards of cyanidin-3-glucoside, phenolic acids (caffeic, ferulic and chlorogenic acid) and Trolox were purchased from Sigma Aldrich, Co. (St. Louis, MO, USA). Extraction chemicals such as methanol, ethyl acetate, citric acid, oxalic acid, and sodium borate along with solvents for chromatography were purchased from Fisher Scientific (San Jose, CA, USA).

2.2. Effect of pre-heating

The effects of pre-heating on the polyphenolic recovery from PSP were evaluated. Randomly selected whole PSP were washed, manually cut into 5 cm cubes, mixed, and divided into five groups for immersion in a deionized water bath (Thermo Scientific SWB25, USA) at 30, 50, 70 and 90 °C for 10 min as a pre-heated treatment and compared to a non-heated control group. Following immersion at each temperature, approximate 5 cm PSP cubes were drained of surface water and shredded in a kitchen-scale food processor for subsequent extraction. Pre-heated PSP treatments were extracted in individual vessels (1:6 w/v ratio) containing water acidified to pH 2.0 with citric acid and held for 1 h in a thermostatic water bath at 70 °C with continuous stirring. Following extraction, treatments were cooled in an ice water bath for 5 min, centrifuged at 4000×*g* for 15 min (Eppendorf Centrifuge 5810R, Eppendorf North America, Westbury, NY, USA), and the supernatant filtered through a 1 cm bed (9 cm diameter) of acid-washed diatomaceous earth under mild vacuum to clarify. Treatments were held frozen at −18 °C until analysis.

2.3. Effect of PPO inhibiting agents

The effect of PPO inhibiting agents and their impact on anthocyanin recovery was evaluated for both pre-heated (90 °C for 10 min) and non-heated PSP. To the aqueous citric acid extraction solution at pH 2.0 that served as a control, the addition of 1% w/v sodium borate, 1% oxalic acid, and 1% citric acid were made. Anthocyanins were likewise extracted at 70 °C for 1 h in a thermostatic water bath and clarified as previously described. The effects of PPO, that can act to co-oxidize anthocyanins, were also evaluated on PSP cubes treated with and without pre-heating at 90 °C and held for 15 min prior to extraction. Activity of residual PPO was determined by homogenizing PSP cubes in 0.05 M phosphate buffer at pH 6.8 and the homogenate filtered and kept in an ice water bath (Jiang, Pen, & Li, 2004). Extracts were then centrifuged at 19,000×g for 20 min at 4 °C and the supernatant used as the enzyme extract. Chlorogenic acid was used as a substrate and enzyme activities immediately measured at 410 nm and again after 24 h of holding the extract at 25 °C.

2.4. Polyphenolic fractions and analysis

Total soluble polyphenolics were characterized directly from the clarified PSP extract while sub-fractions were created by partitioning from 5 g pre-conditioned Sep-Pak columns (Waters Corporation, Milford, MA). Partitioning created a non-anthocyanin polyphenolic fraction that was first eluted with 100% ethyl acetate and a predominantly anthocyanin fraction eluted with 100% methanol containing 0.01% v/v HCl as previously performed (Rodriguez-Saona & Wrolstad, 2001). Solvents were evaporated under vacuum at 35 °C and re-dissolved in a known volume of 0.5 M citric acid buffer at pH 3. Analysis of total anthocyanin content was determined using the pH-differential method (Wrolstad, Durst, & Lee, 2005) and results expressed in equivalents of cyanidin-3-glucoside (mg/kg FW). Total soluble polyphenols representing the total metal ion reducing capacity was determined by the Folin–Ciocalteu assay (Singleton & Rossi, 1965) and data expressed in mg/kg chlorogenic acid equivalents (CAE). The antioxidant capacity of PSP extracts was measured by the oxygen radical absorbance capacity method (ORAC) and the results expressed in μM Trolox equivalents per mL (μM TE/mL) (Ou, Hampsch-Woodill, & Prior, 2001). Individual anthocyanin glycosides and non-anthocyanin phenolic acids were tentatively characterized by HPLC-ESI-MS^n using a Thermo Finnigan LCQ Deca XP Max ion trap mass spectrometer equipped with an ESI ion source run in positive ionization mode (ThermoFisher, San Jose, CA, USA) according to a previous developed method by Pacheco-palencia, Hawken, and Talcott (2007). Following initial compound characterization, routine quantification of anthocyanins was conducted using a Waters 2695 Alliance HPLC system (Waters Corp., Milford, MA) equipped with a Waters 996 photodiode array detector. Separations were conducted using a 250 × 4.6 mm Acclaim 120 C_{18} column (Dionex, Sunnyvale, CA) with a C_{18} guard column. Mobile phases consisted of 10% v/v acetic acid and 5% methanol in water (Phase A) and a 1% acetic and 1% formic acid in methanol (Phase B) run at 0.7 mL/min. A gradient solvent program ran Phase B from 0% to 30% in 3 min, from 30% to 50% in 2 min, from 50% to 70% in 5 min, from 70% to 80% in 2 min, from 80% to 100% in 3 min prior to re-equilibration. Individual anthocyanins were additionally monitored at 520 nm and quantified in mg/kg equivalents of cyanidin-3-glucoside while individual phenolic acids were monitored at 280 nm and quantified in mg/kg of caffeic, ferulic or chlorogenic acid equivalents.

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