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Conventional, ultrasound-assisted, and accelerated-solvent extractions of anthocyanins from purple sweet potatoes

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ABSTRACT

Purple sweet potatoes (PSPs) are rich in anthocyanins. In this study, we investigated the extraction efficiency of anthocyanins from PSPs using conventional extraction (CE), ultrasound-assisted extraction (UAE), and accelerated-solvent extraction (ASE). Additionally, the effects of these extraction methods on antioxidant activity and anthocyanin composition of PSP extracts were evaluated. In order of decreasing extraction efficiency, the extraction methods were ASE > UAE > CE for anthocyanins (218–244 mg/100 g DW) and CE > UAE > ASE for total phenolics (631–955 mg/100 g DW) and flavonoids (28–40 mg/100 g DW). Antioxidant activities of PSP extracts were CE ≈ UAE > ASE for ORAC (766–1091 mg TE/100 g DW) and ASE > CE ≈ UAE for FRAP (1299–1705 mg TE/100 g DW). Twelve anthocyanins were identified. ASE extracts contained more diacyl anthocyanins and less nonacyl and monoacyl anthocyanins than CE and ASE extracts ($P < 0.05$).

1. Introduction

Anthocyanins are flavonoids responsible for the red, purple, violet, and blue colors of fruits, vegetables, and cereals (Mazza & Miniati, 1993). The increasing interest in anthocyanins as food colorants is attributed to the safety concerns of synthetic food color additives (Giusti & Jing, 2007; Wrolstad & Culver, 2012).

Purple sweet potatoes (*Ipomoea batatas* L.), which are rich in anthocyanins, have been grown in China as food and food colorants (Fan, Han, Gu, & Chen, 2008). Anthocyanins from purple sweet potatoes (PSPs) have bioactive effects, including antioxidant (Teow et al., 2007) and anti-inflammatory activities (Zhang et al., 2009). Additionally, PSP anthocyanins attenuate dimethylnitrosamine-induced liver injury in rats (Hwang et al., 2011).

Extraction is a separation technique that affects the yield, quality, and composition of target compounds (Jing & Giusti, 2007; Rodriguez-Solana, Salgado, Dominguez, & Cortes-Dieguez, 2015). An effective extraction method maximizes the extraction of target compounds with minimal degradation and is based on environmental friendly technologies.

The conventional extraction (CE) of anthocyanins, which is based on the bath stirring extraction method, is performed in acidic solutions to obtain the red stable flavylium cation (Santos, Veggi, & Meireles, 2010). Compared to CE, ultrasound-assisted extraction (UAE), which has been used in the extraction of anthocyanins (Lien, Chan, Lai, Huang, & Liao, 2012) and other phenolics (Carrera, Ruiz-Rodriguez, Palma, & Barroso, 2012), have several advantages including reduced processing time and solvent volume. Additionally, UAE has an effective mass transfer and solvent penetration through the disruption of plant cell walls via acoustical cavitation (Rastogi, 2011). Accelerated-solvent extraction (ASE) is a solvent extraction technique that is performed at elevated temperatures and pressure, conditions that improve extraction kinetics and reduce extraction time and solvent volume (Abdel-Aal, Akhtar, Rabalski, & Bryan, 2014; Truong, Hu, Thompson, Yencho, & Pecota, 2012).

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In this study, we investigated the extraction efficiency of anthocyanins from PSPs using CE, UAE, and ASE. The effects of these methods on anthocyanin composition and antioxidant activity were evaluated.

2. Materials and methods

2.1. Materials and reagents

Purple sweet potatoes (PSPs) were provided by Hainan Yedao Ltd. (Hainan, China). Pure standards of gallic acid, quercetin, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein sodium salt, Folin–Ciocalteu reagent (FC reagent), 2,2′-azobis dihydrochloride (AAPH) and 2,4,6-Tripyridyl-s-triazine (TPTZ) were purchased from Sigma–Aldrich (Shanghai, China). All other chemicals and solvents were of the highest commercial grade and were purchased from Anpel (Shanghai, China).

2.2. Anthocyanins extraction

2.2.1. Conventional solvent extraction

PSPs were cut into pieces after wash and prepared in a JJ-2 blender (Jintan, China) into slurry. Ten grams of PSPs slurry were placed into a 250 mL flask containing 100 mL aqueous ethanol (70%, 80%, and 90%, v/v). The orthogonal array design L_9 (3^4) was employed to study effects of temperature (60, 70 and 80 °C), extraction time (90, 120, and 150 min), aqueous ethanol (70%, 80%, and 90%, v/v), and HCl concentration (0.1%, v/v) in Table 1. Then, mixtures were centrifuged in L535R (Xiangyi, China) at 4000 rpm at 4 °C for 15 min and the supernatants were collected. The solution was evaporated using a RE-52 rotary evaporator (Yarong, China) and adjusted to 25 mL with corresponding extraction solutions. The extracts were stored at −18 °C in the dark until analysis. All experiments were conducted in triplicates.

2.2.2. Ultrasound-assisted extraction

Ten grams of PSPs slurry were added into a 250-mL conical bottles containing 100 mL of 80%, 90%, or 100% (v/v) aqueous ethanol solutions. The bottles were then capped and put into a THC ultrasound extraction (Ji Ning Tianhua Ultrasonic Electronics, China). Four three-level factors including temperatures (40, 50, and 60 °C), duration (40, 50, and 60 min), ethanol concentration (80%, 90%, and 100%, v/v) and ultrasonic output power (200, 240, and 280 W) were studied via the Taguchi orthogonal array design L_9 (3^4) in Table 1. Aqueous ethanol solution was acidified by adding 0.1% (v/v) HCl for all UAE experiments. Mixtures were centrifuged at 4000 rpm at 4 °C for 15 min after extraction. The supernatants were collected and adjusted to 25 mL after evaporation. All experiments were conducted in triplicates. The extracts were stored at −18 °C in the dark until analysis.

2.2.3. Accelerated-solvent extraction

The accelerated-solvent extractions was carried out with an ASE 350 Accelerated Solvent Extractor system (Dionex, Sunnyvale, USA). Ten grams of PSPs slurry were added into a 100-mL Zirconium extraction cell (Dionex, Sunnyvale, USA). The extraction cells were arranged in the sample carousel and extracted in a Taguchi L_4 (2^3) design including three two-level factors in Table 1. Factors and levels have been chosen as a result of considering the cost of conducting experiments and single factorial experimental results. The 80% (v/v) aqueous ethanol containing 0.1% (v/v) HCl was used as the extraction solvent for all experiments. The supernatants were collected and adjusted to 25 mL after evaporation. Three trails were conducted for all experiments. The extracts were stored at −18 °C in the dark until analysis.

Table 1
Factors and levels of Taguchi orthogonal array designs.

Design	Methods	Factor (*i*)	Level (*j*)		
			1	2	3
L_9 (3^4)	CE	Temperature (°C)	60	70	80
		Time (min)	90	120	150
		Ethanol (%, v/v)	70	80	90
		HCl (%, v/v)	0.01	0.05	0.1
	UAE	Temperature (°C)	40	50	60
		Time (min)	45	60	75
		Ethanol (%, v/v)	80	90	100
		Power (W)	200	240	280
L_4 (2^3)	ASE	Temperature (°C)	80	90	
		Static time (min)	15	20	
		Static cycle	1	2	

2.3. Total monomeric anthocyanins

The total monomeric anthocyanins in PSPs extracts using different extracted methods were determined by the pH differential method (Giusti & Wrolstad, 2001). An L5S UV–visible spectrophotometer (Shanghai Analytical Instrument, China) was used to read absorbance at 520 and 700 nm. Monomeric anthocyanins content were expressed as cyanidin-3-glucoside equivalents (CGE), using a molecular weight of 449.2 mg/L and the molar absorptivity of 26,900 L cm^{-1} mg^{-1}. Cuvettes with 1-cm path length were used. Measurements were performed in triplicates.

2.4. Total phenolics

Total phenolic content was measured using a modified Folin–Ciocalteu method (Waterhouse, 2001). Briefly, a series of tubes were prepared with 0.5 mL water or samples and 2.5 mL Folin–Ciocalteu reagent for 5 min. After that, 2 mL of 75 g/L Na_2CO_3 solution was added to each test tube and mixed well before incubation at room temperature for 2 h in dark. Then the absorbance of samples and standards was measured at 760 nm in the L5S UV–visible spectrophotometer after zeroing the spectrophotometer with a water blank. Total phenolic content was calculated as gallic acid equivalents (GAE) based on a gallic acid standard curve. Disposable cuvettes of 1-cm path length were used. Each sample was evaluated using three replications.

2.5. Total flavonoids

The total flavonoids content of each PSP extracts was determined using a slightly modified method described previously (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, 5 mL of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of a sample solution and let stand for 10 min. Then, the absorbance of samples or standards was measured at 415 nm in the L5S UV–visible spectrophotometer against a blank sample consisting of a 5 mL methanol solution without $AlCl_3$. The total flavonoids content was calculated using a standard curve with quercetin (0–50 mg/L) as the standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE) per 100 g of PSPs.

2.6. Antioxidant activity assays

2.6.1. Oxygen radical absorbance capacity (ORAC) assay

The oxygen radical absorbance capacity of the extracts from PSPs was investigated according to previously reported procedure (Jing et al., 2014) with a slight modification in an Infinite F200 Pro microplate reader (Tecan, Männedorf, Switzerland). Samples and Trolox standards were prepared with ethanol. All other reagents