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Pressurized liquid extraction and quantification of anthocyanins in purple-fleshed sweet potato genotypes[☆]V.D. Truong^{a,*}, Z. Hu^a, R.L. Thompson^a, G.C. Yencho^b, K.V. Pecota^b^a USDA-ARS, SAA Food Science Research Unit, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC 27695-7624, USA^b Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7624, USA

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ABSTRACT

Analysis of anthocyanins responsible for the purple flesh color is important for breeding programs and development of value-added products. This study aimed to optimize the conditions for anthocyanin extraction from purple-fleshed sweet potatoes (PFSP) using pressurized-liquid extraction (PLE) method and quantify anthocyanins in various genotypes. Freeze-dried powders of PFSP genotypes were extracted with acidified methanol using an accelerated solvent extractor. Anthocyanin content of the extract was characterized by (a) pH-differential method and (b) color value protocol measuring absorbance at 530 nm, which is commonly used in Japan as a commercial indicator of total anthocyanin quantity. Highest anthocyanin yields by PLE were with an acetic acid:methanol:water mixture of 7:75:18% (v/v), sample weight of <0.5 g and 80–120 °C. Among 335 genotypes, the anthocyanin content varied widely from 0 to 663 mg cyanidine-3-glucoside equivalent/100 g powder or 0–210 mg/100 g fresh weight. The total monomeric anthocyanin (TMA) contents determined by the pH-differential method were highly correlated with the Japan color value (JCV) protocol, $TMA = (0.145) JCV$, $R^2 = 0.943$. These results can be useful for sweet potato breeding programs and processing industry in development of PFSP cultivars and processed products with high anthocyanin levels.

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1. Introduction

Sweet potato (*Ipomoea batatas* L.) cultivars with deep purple flesh have been developed in many countries to meet a growing demand in the markets for health foods. Purple-fleshed sweet potatoes (PFSP), such as the Yamagawamurasaki and Ayamurasaki cultivars, were developed in Japan and are utilized in a variety of processed commercial products including natural food colorants, purple paste and flour which are used in juices, bread, noodles, jams, confectionary, and fermented beverages (Suda et al., 2003; Yamakawa and Yoshimoto, 2002). Recent research on nutraceutical properties of PFSP indicated that the extracted anthocyanins exhibited strong radical scavenging activity, anti-mutagenic activity, and significantly reduced high blood pressure and liver injury (Kano et al., 2005; Suda et al., 2008; Zhang et al., 2009). Other physiological functions of anthocyanins include anti-inflammatory activity, antimicrobial activity, ultraviolet light protection, and reduction in memory impairment (Suda et al.,

2003; Wu et al., 2008). In the United States, there is a growing interest in the sweet potato industry in exploring these opportunities for PFSP in the fresh markets and processing industry. Breeding efforts to develop PFSP genotypes suitable for local growing conditions, postharvest handling practices and marketing systems have been carried out at North Carolina State University (NCSU). Processing technologies have been developed to convert PFSP into frozen and aseptic purees for various food applications (Steed and Truong, 2008; Steed et al., 2008).

In quantification of anthocyanins in numerous samples generated from the breeding efforts and various studies on nutraceutical properties, chemical characterization and product quality, anthocyanin extraction is a critical step of sample preparation. Conventionally, the methods employed in extraction of anthocyanins and other phenolic compounds from fruits and vegetables, including sweet potatoes, involve blending the materials with a water–organic solvent mixture containing acetone, ethanol and methanol for various lengths of time, from 15 min to several hours (Robbins, 2003). The optimum temperature, time and solid–solvent ratio for extraction of PFSP anthocyanins were determined at 80 °C, 60 min, and a solid–solvent ratio of 1:32 (Fan et al., 2008). In Japan, a standard procedure for spectrophotometric measurement of color value as a commercial indicator of anthocyanin content in PFSP involves extracting anthocyanins by soaking PFSP particulates in 50% acetic

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acid for up to 16 h (Konczak-Islam et al., 2003; Yoshinaga et al., 1999). These methods are time consuming and use large amounts of organic solvents.

Pressurized-liquid extraction (PLE), which is also known as accelerated solvent extraction (ASE), applies pressure and elevated temperature with liquid solvents to achieve fast and efficient extraction. The use of PLE can improve sample throughput by reducing extraction time and minimizing or eliminating the use of toxic solvents (Bjoerklund et al., 2000). PLE has been used as an innovative technique in the extraction of anthocyanins from other fruits and vegetables including spinach, berries and grape pomace (Howard and Panjaitan, 2008; Monrad et al., 2010). However, limited work has been reported in the application of PLE in polyphenolic extraction of starchy vegetables such as sweet potatoes. Rabah et al. (2005) reported the use of pressurized water at high temperatures of 200–300 °C as an effective technique in producing the phenolic sweet potato extracts with high antioxidant activity and cytotoxic and apoptosis induction activities on the H-60 leukemia cell line. Recently, PLE has been used in anthocyanin extraction from freeze-dried powders of PFSP for quantification, antioxidant activity assays and HPLC profiles (Steed and Truong, 2008; Truong et al., 2010). Yet, optimization of PLE conditions affecting anthocyanin extraction has not been reported.

In this study, the factors affecting the efficiency of anthocyanin extraction from starchy PFSP, namely water–solvent mixture, temperature and sample weight, were determined using response surface methodology (RSM). Anthocyanins content of the extracts from various clones were determined using the pH-differential method (Giusti and Wrolstad, 2001; Lee et al., 2005) as related to the color value protocol that have been reported in various reports on PFSP research in Japan (Konczak-Islam et al., 2003; Yoshinaga et al., 1999).

2. Materials and methods

2.1. Chemicals

HPLC grade methanol (99.9%) was purchased from Sigma–Aldrich (St. Louis, MO). Water used for HPLC analysis was purified with a deionized water system (Pure Water Solutions, Hillsborough, NC, USA). All other chemicals were analytical grade (Fisher Scientific, Suwanee, GA, USA).

2.2. Sweet potato genotypes and sample preparation

Sweet potato clones with varying flesh colors (white, cream, yellow, orange, purple with cream, cream with purple, medium purple and dark purple) were the F1 generation from a 5 × 5 North Carolina (NC) Design II mating design of the Sweet potato Breeding Program at NC State University. The clones were identified based on the family followed by a selection number (e.g. 11–5, 12–17, 13–18), and grown at the experimental fields in Clinton, NC, USA. The harvested roots of 335 genotypes were cured 30 °C, 85–90% relative humidity for 7 days and stored at 13–16 °C and 80–90% relative humidity for 2–3 months before sampling for analysis. Three roots (400–1500 g) per clone were peeled and cut into 0.5 cm thick pieces. Samples (100 g) were taken, placed in plastic bags, and immediately placed in a –20 °C freezer. A portion of each of the sliced samples were chopped into fine pieces and placed in an oven at 70 °C for 72 h for moisture determination. All the frozen samples were freeze-dried for 4–6 days in the dark using a VirTis Genesis 25XL freeze dryer (Gardiner, NY, USA) operated at –35 °C. The freeze dried samples were weighed and ground into powder using a Mr. Coffee® precision coffee grinder (Sunbeam Boca Raton,

FL, USA), placed in sample vials and kept in –20 °C storage until analysis.

2.3. Colorimetric measurement of sweet potato powders

The color of the freeze-dried powders was measured using a tristimulus colorimeter (Model D25/DP9000, Hunter Associate Laboratories Inc., Reston, VA, USA) and expressed as L^* , a^* , b^* values. The instrument (45°/0° geometry, D25 optical sensor) was calibrated against a standard white tile ($L^* = 92.75$, $a^* = -0.76$, $b^* = -0.07$). Samples were filled into a 35 mm petri dish, covered, and carefully pressed against the surface to remove air bubbles. Three measurements were taken on each sample and values for L^* , a^* , and b^* were averaged. Hue angle (H^*) was calculated as $\arctan(b^*/a^*)$ as described by Hutchings (1994).

2.4. Conventional pigment extraction and Japan color value of extracts (JCV)

Anthocyanins in sweet potato powders were extracted following the standard method of the Japan Food Additives Association (Konczak-Islam et al., 2003; Yoshinaga et al., 1999). One gram of sample was vortexed for 1 min in 20 mL of 50% acetic acid, and incubated for 16 h at room temperature in the dark. The extract was centrifuged at 10,000 rpm for 10 min, diluted fourfold with McIlvaine's buffer solution (pH 3.0) and the absorbance measured at 530 nm (OD_{530}) with a Varian spectrophotometer (Cary WinUV Model 300, Palo Alto, CA, USA). Color value of the pigment extract (JCV), which is a Japan commercial indicator of total anthocyanins, was calculated using the following formula: $JCV = 0.1 \times OD_{530} \times 4 \times 20/1$ g powder.

2.5. Pigment extraction by accelerated solvent extractor

Extraction of polyphenolic compounds from freeze dried sweet potato powders was performed using an accelerated solvent extractor, ASE 200 (Dionex Corporation, Sunnyvale, CA, USA) equipped with a solvent controller. Three cellulose filters were placed in the bottom of a 22 mL stainless steel extraction cell and covered with 26 g of acid-washed sea sand (Fisher Scientific, Pittsburgh, PA, USA). Sweet potato powder was mixed with the sea sand, loaded into the cell, and then closed tightly. Extraction was carried out at a constant pressure of 1500 psi using aqueous solvent with varying concentrations of methanol/water (% v/v). Unless otherwise specified, the acid concentration in the extraction solvents was kept at a constant level of 7% acetic acid (hereafter referred to as acidified methanol). The solvents were degassed by flushing with nitrogen gas for 1 h before use. Optimal methanol levels, sample weight and extraction temperature were determined using response surface methodology. Based on preliminary experiments, the following conditions of ASE 200 were operated with no preheating time, a 5 min heating time, three 5 min cycles, flushing volume at 60% of the sample cell volume and a nitrogen purge time of 60 s. Extracts were collected in amber glass vials, adjusted to 50 mL volume with solvent, and then transferred into several 10 mL serum tubes and kept at –80 °C until analysis.

2.6. Quantification of total monomeric anthocyanins

Total monomeric anthocyanin content was determined using the pH-differential method (Giusti and Wrolstad, 2001; Lee et al., 2005). Two dilutions were performed on each sample. The first used potassium chloride (0.025 M) at pH 1 and the second was with sodium acetate (0.4 M) at pH 4.5. Samples were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 min before absorbance at 530 and

700 nm were recorded using a spectrophotometer calibrated with distilled water as the blank.

The difference in absorbance between pH values and wavelengths was calculated:

$$A = (A_{530\text{ nm}} - A_{700\text{ nm}})_{\text{pH}1.0} - (A_{530\text{ nm}} - A_{700\text{ nm}})_{\text{pH}4.5}$$

The concentration of monomeric anthocyanin pigment was obtained:

Monomeric anthocyanin pigment (mg/L) = $(A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times 1)$ where MW is the molecular weight, DF is the dilution factor, ϵ is the molar absorptivity, and 1 is for a standard 1 cm path length. The molecular weight (MW = 449.2 g mol⁻¹) and molar absorptivity ($\epsilon = 26,900 \text{ L cm}^{-1} \text{ mol}^{-1}$) for cyanidin-3-glucoside was used. Total monomeric anthocyanins were reported as milligrams anthocyanins per 100 g fresh weight or dry weight (mg cyanidin-3-glucoside/100 g fw or dw).

2.7. HPLC analysis

The anthocyanin profiles of the extracts generated after RSM optimization were analyzed by HPLC-DAD method as previously described (Truong et al., 2010). The HPLC system was equipped with a UV6000LP photodiode array detector, AS3000 autosampler, SCM1000 degasser, P2000 binary pump and ChromQuest software version 4.1 (Thermo Electron Corp., San Jose, CA, USA). The column used was a 250 mm × 2 mm i.d., 4 μm, Synergi Polar-RP C18 (Phenomenex, Torrance, CA, USA) attached to a guard column of 7.5 mm × 4.6 mm i.d., Allsphere Phenyl 5 μm (Alltech, Deerfield, IL, USA). The operating conditions were as follows: autosampler sample tray temperature at 6 °C; column oven temperature at 35 °C; injection volume was 10–20 μL; and eluent flow rate was 0.23 mL/min. The elution solvents were A (1%, v/v formic acid in water) and B (acetonitrile containing 1% formic acid) with the following gradient: 5–45% B from 0 to 50 min, 45–5% B from 50 to 55 min, and isocratic at 5% B from 55 to 60 min to equilibrate the column for the next injection. Spectral data from 200 to 600 nm were recorded, and the anthocyanin chromatograms were monitored at 530 nm. Identification of the major anthocyanin compounds was based on the retention time as described by Truong et al. (2010).

2.8. Experimental design and statistical analysis

Response surface methodology was used to investigate the optimum combination of sample weight (NC415 genotype),

methanol concentration and operating temperature on extraction of anthocyanins from the freeze-dried powders. A 3-factor, 3-level, face-centered cube design was employed which consisted of 18 experimental runs, including 4 replicates at the center point (Myers and Montgomery, 2002). All 18 treatment combinations are shown in Table 1. The independent design variables were acidified methanol level, X_1 (0, 25, 50, 75 and 100%), extraction temperature, X_2 (20 °C, 50 °C, 80 °C, 110 °C and 140 °C), and sample weight, X_3 (0.2, 0.8, 1.4, 2.0 and 2.6 g), while the response variable was the concentration of anthocyanins in the extracts. This design examined differences between the treatments and generated a model for the response surface to predict how the independent variables will influence the extraction of anthocyanins from the freeze-dried sweet potato samples.

The response surface regression procedure of the statistical analysis system (SAS, Release 8.02, Cary, NC, USA) was used to analyze the experimental data and obtain regression coefficients. The data were fit to a full second order polynomial equation as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1^2 (X_1)^2 + \beta_2^2 (X_2)^2 + \beta_3^2 (X_3)^2 + \beta_{12} (X_1 X_2) + \beta_{13} (X_1 X_3) + \beta_{23} (X_2 X_3) + \text{error} \quad (1)$$

where Y = response of anthocyanins in the extracts, β_0 = intercept, β_1 , β_2 , and β_3 = the regression coefficients for the linear terms, β_1^2 , β_2^2 , β_3^2 = quadratic terms, β_{12} , β_{13} , β_{23} are the interaction terms.

For other analyses, the experiment was conducted with two replicates in a randomized complete block design. Group differences were evaluated using t -tests with $p < 0.05$ considered to be a statistically significant difference. Means were compared with Duncan's multiple range test with $\alpha = 0.05$, Pearson correlations and non-linear regression were performed using SAS Statistical Analysis System (v. 8.1; SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Extraction of anthocyanins

Acidified solutions of ethanol, methanol, acetone, water and mixtures of these solvents have been used to extract anthocyanins from various food materials and agricultural wastes. Hydrochloric acid, trifluoroacetic acid or weak organic acids such as acetic acid, citric acid, lactic acid, malic acid and tartaric acid have been used to maintain a low pH in the extraction of anthocyanins from fruits

Table 1
Experimental design on optimization of pressurized liquid extraction conditions and total monomeric anthocyanin (TMA) results.

Design point	Coded levels			Actual levels			Measured TMA (mg/g powder)
	X_1	X_2	X_3	Methanol X_1 (%)	Temperature X_2 (°C)	Sample weight X_3 (g)	
1	-1	-1	-1	25	50	0.8	1.32 ± 0.04
2	-1	-1	1	25	50	2	1.27 ± 0.02
3	-1	1	-1	25	110	0.8	1.24 ± 0.01
4	-1	1	1	25	110	2	0.85 ± 0.01
5	1	-1	-1	75	50	0.8	1.40 ± 0.01
6	1	-1	1	75	50	2	1.37 ± 0.02
7	1	1	-1	75	110	0.8	1.54 ± 0.03
8	1	1	1	75	110	2	1.45 ± 0.01
9	-2	0	0	0	80	1.4	0.82 ± 0.07
10	2	0	0	100	80	1.4	1.45 ± 0.02
11	0	-2	0	50	20	1.4	1.24 ± 0.06
12	0	2	0	50	140	1.4	1.16 ± 0.04
13	0	0	-2	50	80	0.2	1.52 ± 0.03
14	0	0	2	50	80	2.6	1.25 ± 0.01
15	0	0	0	50	80	1.4	1.33 ± 0.02
16	0	0	0	50	80	1.4	1.33 ± 0.05
17	0	0	0	50	80	1.4	1.36 ± 0.03
18	0	0	0	50	80	1.4	1.38 ± 0.04

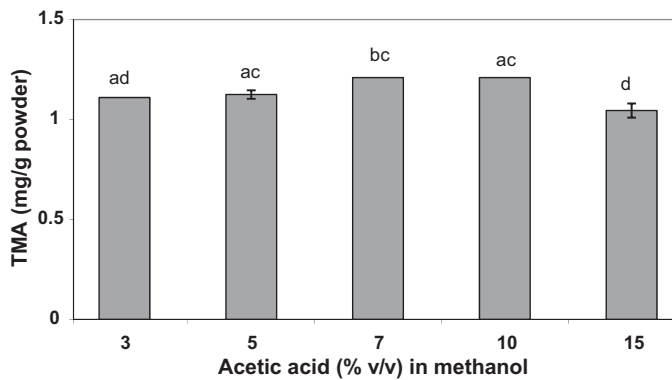


Fig. 1. Anthocyanin content of ASE extracts as affected by acetic acid levels in the solvent. Extraction conditions: 0.5 g sample, 50 °C, 1500 psi. Identical letters over the bars indicate that there was no significant difference between treatments ($p \geq 0.05$).

and vegetables including PFSP (Ju and Howard, 2003; Rhim, 2002). The acidified solvents denature cellular membranes and facilitate the solubilization of anthocyanins from the pigmented globules described as anthocyanoplasts in vacuoles of the plant cells (Andersen and Jordheim, 2006; Nozue et al., 2003). Though the acids provide a favorable condition for the formation of flavylum ions and the stabilization of anthocyanins, addition of excess acid to the extraction solvents may result in degradation of the native form and lead to important changes in total anthocyanin content and artifacts in pigment components of the extracts (Bakker and Timberlake, 1985; Revilla et al., 1998). Acetic acid concentration up to 50% has been used in Japan to determine the anthocyanin content in PFSP (Yoshinaga et al., 1999). This level of acetic acid in the solvent seems to be too high since our results showed that the yield of total monomeric anthocyanins in terms of mg TMA per gram of PFSP powder significantly declined at 15% acetic acid in methanol ($p \leq 0.05$) while the highest yields were obtained at 5–10% acetic acid (Fig. 1). A 7% acetic acid in methanol was used in extraction of anthocyanins from PFSP by Philpott et al. (2003) and grape skin by Ju and Howard (2003).

The 7% acetic acid level was used to maintain a pH of around 2.4 in the acidified solvents in the subsequent experiments to determine the effects of acidified methanol concentration, temperature and sample weight on TMA yields from PFSP powders. The treatment combinations and the mean values of TMA yields are shown in Table 1. Multiple regression analysis resulted in the following full model ($p \leq 0.01$, $R^2 = 0.971$, coefficient of variation = 3.7%) for the three independent variables:

$$\begin{aligned} \text{TMA} = & 1.19542 + 0.00145X_1 + 0.00388X_2 - 0.06938X_3 \\ & - 0.00008X_1^2 + 0.00012X_1X_2 - 0.00004X_2^2 \\ & + 0.00238X_1X_3 - 0.00301X_2X_3 + 0.02697X_3^2 \end{aligned} \quad (2)$$

where X_1 = % acidified methanol, X_2 = temperature (°C), X_3 = sample weight (g).

The linear, quadratic and interaction terms were all significant ($p \leq 0.01$, $R^2 = 0.97$) indicating that the three independent variables, percentage of acidified methanol in the extraction solvent (X_1), temperature (X_2) and sample weight (X_3), had an influence on anthocyanin extraction efficiency. The full model was used to maintain model hierarchy and to generate the graphs of TMA as a function of sample weight and acidified methanol (Fig. 2), and acidified methanol and temperature (Fig. 3). Positive linear coefficients and negative quadratic coefficients of X_1 and X_2 resulted in a curvilinear effect in anthocyanin yield. For the ranges of acidified methanol and temperature used in the extraction

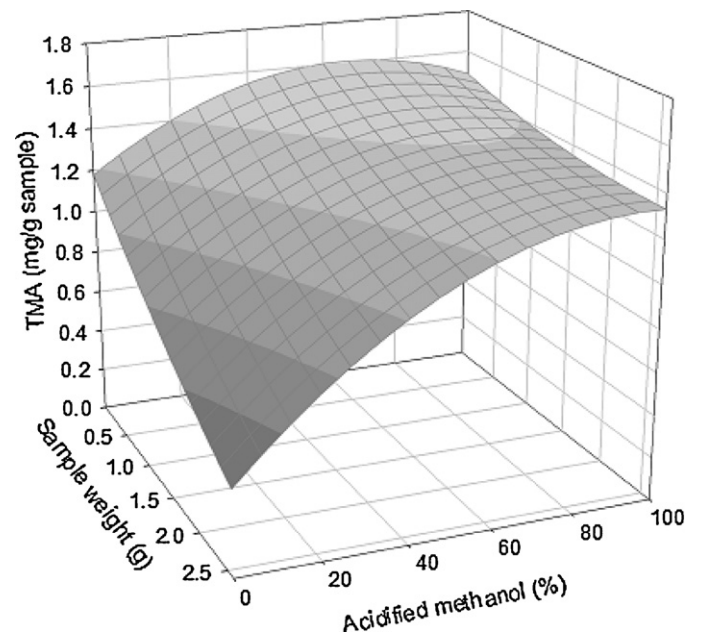


Fig. 2. Response surface of extracted TMA at constant temperature of 100 °C as a function of sample weight and acidified methanol concentration.

experiments, sample weight had a strong effect on the anthocyanin yields, and its negative linear coefficient indicated a decrease in anthocyanin yield with increasing sample weight. The response surface and contour plot in Fig. 2 indicates that sample weights of less than 0.5 g powder should be used for maximizing TMA extraction from PFSP powder using the 22 mL extraction cell of ASE. With regard to extraction temperature, Fig. 3 shows that the optimum temperature for ASE extraction was beyond 140 °C that was set as an upper limit for the designed experiment. Within the temperature range chosen in this study, the optimum level of methanol was in a range of 73–87% in the solvent mixture containing 7% acetic acid, methanol and water. The water–methanol solvents have moderate solvent polarity, therefore, they are more efficient in degrading the plant cell walls which have low

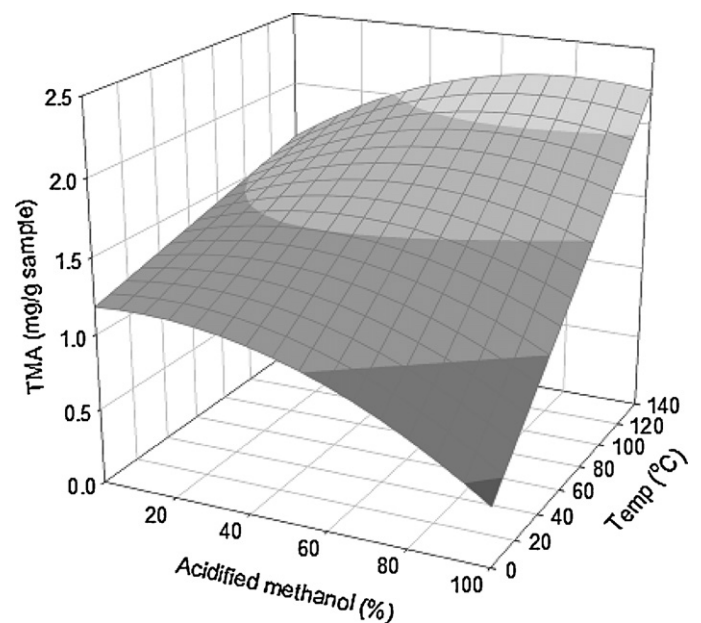


Fig. 3. Response surface of extracted TMA at constant sample weight of 0.25 g as a function of acidified methanol concentration and temperature.

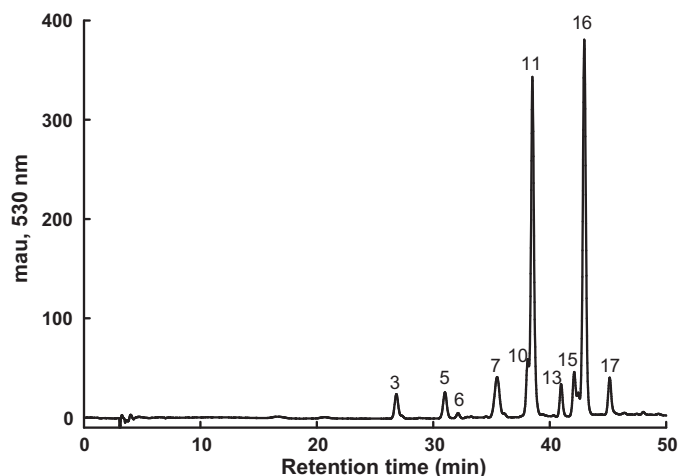


Fig. 4. Typical HPLC chromatogram of PFSP anthocyanin extracts. ASE extraction conditions: 0.5 g PFSP powder, 80% acidified methanol, 1500 psi and 80 °C. Peaks were numbered and identified as previously reported (Truong et al., 2010): peak 3, cyanidin-3-p-hydroxybenzoylsophoroside-5-glucoside; peak 5, peonidin 3-p-hydroxybenzoylsophoroside-5-glucoside; peak 6, cyanidin 3-(6''-feruloylsophoroside)-5-glucoside; peak 7, cyanidin 3-(6''-caffeoylsophoroside)-5-glucoside; peak 10, cyanidin 3-(6''-6''-dicafeoylsophoroside)-5-glucoside; peak 11, peonidin 3-caffeoylsophoroside-5-glucoside and cyanidin 3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside; peak 13, peonidin-dicafeoylsophoroside-5-glucoside; peak 15, unknown; peak 16, peonidin 3-caffeoyl-p-hydroxybenzoyl-sophoroside-5-glucoside; peak 17, peonidin-caffeoyl-feruloylsophoroside-5-glucoside.

polarity and therefore facilitate the release of anthocyanins and other polyphenols from the cells. Liu et al. (2000) reported that solvents with very high polarity, such as water, or very low solvent strength, such as chloroform and hexane, did not result in good extraction of bioactive compounds from the medicinal herb, *Hypericum perforatum*. These authors stated that water–ethanol, ethanol–acetone and acetone had more extraction capabilities, and 44–69% ethanol in acetone at 55 °C was found as optimum extraction conditions. However, we observed that the PFSP extracts

from the treatments with low methanol levels (0, 25 and 50%) and high extraction temperatures (≥ 50 °C) had foams and precipitates which may be attributable to the extracted water soluble substances such as proteins, pectic substances and other polysaccharides. The extracts from the treatment combinations of low sample weight, high percentage of acidified methanol (70% and 100%) and temperature of greater than 80 °C had none or little precipitates and foam formation. The HPLC–DAD profile of anthocyanins extracted from 0.5 g PFSP powder in 80% acidified methanol with ASE operated at 100 °C is shown in Fig. 4. Similar chromatograms were obtained for the extracts performed at 80 °C and 120 °C (data not shown). The profile had the same anthocyanin components for NC415 genotype as previously reported (Truong et al., 2010), and the peak identification is shown in the caption of Fig. 4.

Therefore, it is recommended that optimum conditions for extracting anthocyanins from PFSP powders using ASE 200 equipped with a 22 mL extraction cell are: 75% methanol in acidified water–methanol solvent, temperature of 80–100 °C and sample weight of less than 0.5 g powder. The predicted model (Eq. (2)) was verified at a constant sample weight of 0.5 g PFSP powder, solvent of 75% acidified methanol and temperature of 80–100 °C. The predicted TMA values agreed well with the measured TMA content, and the percentage of predicted and measured TMA values was in a range of 91–107% (data not shown), indicating the relative reliability of the model. These recommended conditions are in accordance with previous reports where 100–120 °C were identified as optimum temperatures for PLE of anthocyanins and other phenolics from various plant materials such as fruit pomaces, grape skin, berries, spinach and eggplant (Howard and Panjaitan, 2008; Ju and Howard, 2003; Luthria and Mukhopadhyay, 2006). Higher temperatures (130–170 °C) are more effective in PLE of flavonoids and antioxidant-rich moieties such as Maillard polymers from spinach and red grape skin using 70% ethanol and subcritical water, respectively, but temperatures of higher than 100 °C may cause degradation of total anthocyanins (Howard and Panjaitan, 2008). The extraction temperature of 80 °C was recommended as an optimum level in a study on optimizing the conditions for conventional extraction of anthocyanins from PFSP using acidified ethanol (Fan et al., 2008).

Table 2

Dry matter, total monomeric anthocyanin (TMA) content, Japan color value (JCV) and Hunter color characteristics of purple-fleshed sweet potato genotypes.

Genotype	Dry matter (%)	TMA (mg/g) (dry weight)	TMA (mg/g) (fresh weight)	Japan color value			Hunter color parameters		
				JCV/g (dry weight)	JCV/g (fresh weight)	L*	a*	b*	Hue (°)
07–19	39.46	2.35	0.93	18.47	7.29	45.13	20.33	–9.18	335.70
18–19	25.00	5.68	1.42	33.95	8.49	40.70	19.51	–12.95	326.43
08–21	31.66	6.63	2.10	46.41	14.69	36.31	24.15	–10.07	337.36
08–01	43.52	1.95	0.85	16.40	7.14	45.89	18.13	–5.46	343.24
08–02	39.50	0.81	0.32	4.75	1.88	60.77	12.67	0.60	2.71
17–02	44.44	2.51	1.12	16.75	7.44	44.89	16.29	–6.93	336.95
08–12	29.94	5.84	1.75	42.94	12.86	39.56	22.84	–9.68	337.03
08–04	39.76	2.78	1.10	23.19	9.22	42.10	21.01	–7.55	340.23
01–11	35.31	0.05	0.02	0.43	0.15	77.64	3.19	11.82	74.90
12–15	36.13	0.90	0.32	6.16	2.23	59.76	17.09	–4.59	344.97
02–19	23.71	0.71	0.17	5.36	1.27	63.34	12.92	–1.00	355.57
12–16	30.38	3.02	0.92	22.06	6.70	45.84	22.85	–12.05	332.19
10–20	35.52	1.58	0.56	11.32	4.02	52.99	17.91	–5.16	343.93
06–03	42.29	0.54	0.23	4.82	2.04	59.49	12.00	–0.67	356.80
03–14	28.11	3.58	1.01	21.30	5.99	46.38	16.82	–9.98	329.32
17–20	28.49	4.38	1.25	21.98	6.26	45.38	18.32	–10.67	329.78
03–13	35.02	3.32	1.16	21.98	7.70	46.57	16.79	–8.69	332.64
03–10	34.95	2.22	0.78	15.91	5.56	52.18	15.09	–8.92	329.41
07–01	40.01	2.10	0.84	15.58	6.23	45.56	20.42	–4.61	347.28
06–16	37.92	1.34	0.51	12.35	4.68	52.44	17.42	–7.45	336.85
13–04	29.66	1.69	0.50	10.53	3.12	53.01	18.29	–5.27	343.93
03–06	34.25	0.31	0.11	3.00	1.03	69.07	6.65	4.57	34.50
12–10	34.31	1.09	0.37	10.65	3.66	53.69	18.51	–10.57	330.27

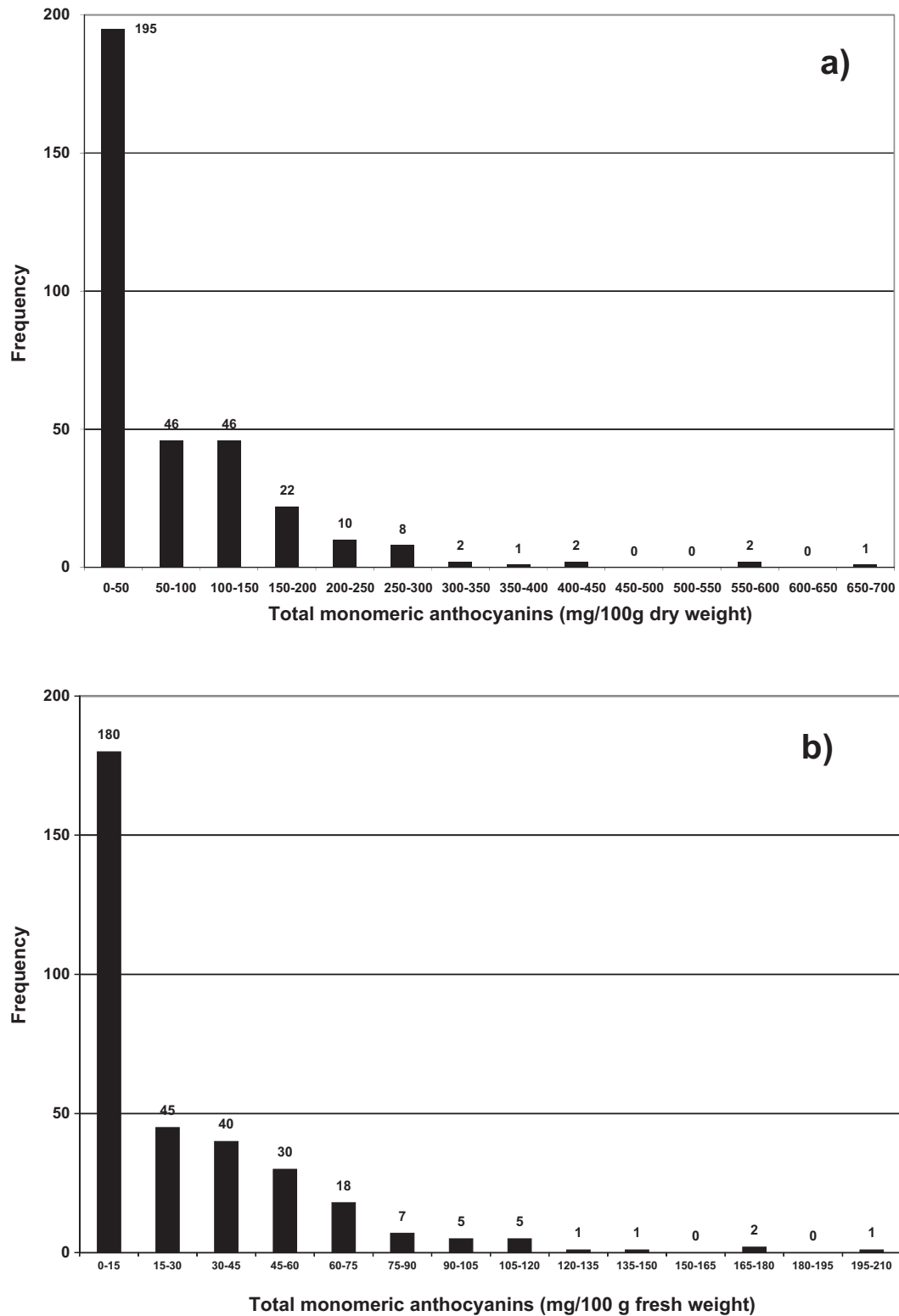


Fig. 5. Frequency distribution of anthocyanin content of 335 sweet potato genotypes on (a) dry weight basis and (b) fresh weight basis.

3.2. Anthocyanins content in various sweet potato genotypes

The optimum conditions for ASE extraction of anthocyanins from PFSP described above were used for determining TMA content of 335 sweet potato genotypes from the f1 generation of the 5 × 5 mating design. The dry matter content of the genotypes ranged from 20 to 46% with a mean of 35%. Table 2 contains part of

the dry matter data. The frequency distribution of TMA contents of 335 genotypes analyzed is presented in both dry weight (Fig. 5a) and fresh weight basis (Fig. 5b). The genotypes differed widely in their anthocyanin contents. The TMA contents ranged from 0 to 663 mg/100 g dry powder with a mean and a median of 71 and 38 mg/100 g dry powder, respectively. Most of the genotypes (85%) contained <150 mg TMA/100 g dry powder, 12% genotypes had

150–300 mg TMA/100 g powder and about 3% of the genotypes had >300 mg TMA/100 g powder. On the fresh weight basis, the TMA contents were in a range of 0–210 mg/100 g with mean and median of 25 and 13 mg/100 g, respectively. About 80% of the genotypes had <50 mg TMA/100 g, 16% contained 50–100 mg TMA/100 g, and about 4% of the genotypes contained >100 mg TMA/100 g. In comparison, anthocyanin contents of 2–40 mg TMA/100 g fresh weight were reported in various genotypes of red-fleshed potatoes (Rodriguez-Saona et al., 1998).

The sweet potato genotypes with an anthocyanin content of >150 mg/100 g fresh weight (>400 mg/100 g dry weight) had a very intense purple-flesh color. Commercial cultivars of PFSP that are grown in the United States contain <100 mg anthocyanins/100 g fresh weight. The cultivars Okinawa and Luna with light purple-flesh color contain 20 and 10 mg TMA/100 g fresh weight while Terlaje and Stokes Purple with medium to dark purple contain 40 and 80–100 mg TMA/100 g fresh weight, respectively (Steed and Truong, 2008; Teow et al., 2007; Truong et al., 2010; Yang and Gadi, 2008). Cevallos-Casals and Cisneros-Zevallos (2003) reported an anthocyanin content of 182 mg TMA/100 g fresh weight in a red-fleshed sweet potato variety from the Andean region. Several purple-fleshed sweet potato cultivars in Japan were reported to contain 5.3–60.0 mg anthocyanins/100 g fresh weight (Suda et al., 2003).

The anthocyanin content of the sweet potato genotypes in this study were lower than those reported for black currant and blueberries (322–476 mg/100 g fw), but they were comparable with other commodities such as grapes (27–120 mg/100 g fw), plum (19–124 mg/100 g fw), sweet cherries (122 mg/100 g fw), raspberries (92 mg/100 g fw), eggplant (86 mg/100 g fw), and red radishes (100 mg/100 g fw) (Wu et al., 2006). The results indicated that many PFSP genotypes fall in the middle of the spectrum of high anthocyanin fruits and vegetables, and there is potential for further increasing the anthocyanin levels in PFSP through breeding and selection of genotypes.

3.3. TMA contents and Japan color values (JCV)

Among the samples analyzed, the freeze-dried powders of 23 genotypes with varied color, from light to dark purple, were subjected to anthocyanin quantification by pH-differential method and the Japan color value (JCV) procedure. The Hunter color values (L^* , a^* , b^* and hue) of these powders ranged from 2.71 to 347.28° (Table 2) indicating a color variation of orange to purple. The purple powder of Ayamurasaki, a popular PFSP cultivar in Japan, had the values of $L^* = 44.0$, $a^* = 21.6$, $b^* = -6.7$ and hue = -0.3 radian = 342.8° (Oki et al., 2002), which were within the ranges of the Hunter color values for the genotypes in this study. For the pH-differential method, the powder samples were extracted at 100 °C by following the ASE procedure, and TMA content was measured as described above. The pH-differential assay is a commonly used method by researchers and the food industry for quantifying total monomeric anthocyanins in many fruits and vegetables. This method has been validated in several laboratories and is being considered by AOAC as the standard method for anthocyanin quantification (Lee et al., 2005). High correlation ($R^2 \geq 0.925$) between the pH-differential method and HPLC analysis in determining anthocyanin content in fruit juices was reported by Lee et al. (2008). In Japan, the color value calculated from the absorbance of the extract at 530 nm has been used as a commercial indicator of total anthocyanin quantity in PFSP and processed products. The sample was extracted with 50% acetic acid overnight, at room temperature and the centrifuged supernatant was diluted with buffer at pH of 3.0 for absorbance reading. The TMA content and JCV of the 23 powdered samples were converted into wet weight based on the dry matter content of the respective genotype.

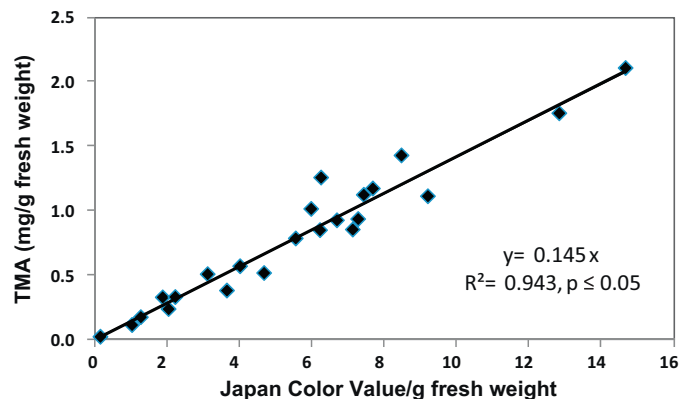


Fig. 6. Correlation between anthocyanin contents determined by pH-differential method and JCV of PFSP clones.

As shown in Table 2, the results ranged from 0.02 to 2.10 mg TMA/g fw and from 0.15 to 14.69 JCV/g fw. These JCV results were comparable with the color values of 1.3–14.5 per gram of raw roots of 19 PFSP clones developed in Japan (Yoshinaga et al., 1999).

A linear relationship with high correlation ($R^2 = 0.951$) between TMA content and JCV per gram of fresh weight of PFSP is shown in Fig. 6. An equation of $TMA = (0.145) JCV$ with $R^2 = 0.950$ was obtained for the dry weight data. Statistical analysis indicated that the intercept term had no significant contribution to the model fitting and it was omitted for both fresh and dry weight data. This simple linear relationship would be useful in comparing the anthocyanin data from various reports on PFSP genotypes, pigmented extracts and other processed products using either pH-differential method or the JCV protocol. With this conversion factor, the PFSP clones with 1.3–14.5 JCV reported by Yoshinaga et al. (1999) had the estimated TMA range of 18.9–210.3 mg cyanidin-3-glucoside/100 g fresh weight which are similar to that of the breeding genotypes analyzed in this study.

4. Conclusions

The results illustrate that the optimal conditions for PLE of anthocyanins from PFSP using ASE 200 were an acetic acid:methanol:water mixture of 7:75:18% (v/v), a sample weight of <0.5 g, and a temperature of 80–100 °C. A wide range of anthocyanin content exists among the PFSP genotypes with a majority falling in the middle of the spectrum of high anthocyanin fruits and vegetables. There is potential for further increasing the anthocyanin levels in PFSP through breeding efforts. The conversion factor between TMA content determined by pH-differential method and JCV shown in this study would facilitate the comparison of total anthocyanin content among the breeding programs.

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