Acid Inhibition on Polyphenol Oxidase and Peroxidase in Processing of Anthocyanin-Rich Juice and Co-product Recovery from Purple-Fleshed Sweetpotatoes

An N. Truong, Yiwen Thor, G. Keith Harris, Josip Simunovic, and Van-Den Truong ២

Abstract: With high phytochemical and starch contents, purple-fleshed sweetpotatoes (PFSP) have been processed into various functional ingredients and food products including juices and natural colorants. For juice processing, PFSP are usually subjected to heat treatment for inactivation of pigment-degrading enzymes. However, heating of sweetpotatoes gelatinizes starch and produces thick slurry with cooked flavor, which are the drawbacks. Development of alternative processes to overcome the stated problems will be beneficial to sweetpotato processors. This study demonstrated that acidified water (\geq 3% w/v citric acid) was effective in inhibiting polyphenol oxidase and peroxidase in raw PFSP resulting in an attractive reddish juice. About 93% total phenolics (TP) and 83% total monomeric anthocyanins (TMA) in PFSP were extracted by two repeated extractions. The combined PFSP juice (3.2 L/kg PFSP) had high levels of TP (1,850 mg/L) and TMA (475 mg/L). With the developed process, 167 g dried starch, and 140 g dried high-fiber pomace were obtained for each kg raw PFSP, besides the highly pigmented juice. Pasteurization of the PFSP juice samples (pH 3.2) at 80 °C for 12 s resulted in 15% loss in TMA and had no effect on TP. The results indicated an efficient process to produce sweetpotato juice with high bioactive compounds and recovery of starch and high dietary fiber pomace as co-products.

Keywords: dietary fiber, Ipomoea batatas, polyphenolics, starch, sweetpotato juice processing

Practical Application: Purple-fleshed sweetpotatoes (PFSP) are rich in polyphenolics and antioxidant activities. In PFSP juice extraction, heat treatment to inactivate the pigment-degrading enzymes results in starch gelatinization and cooked flavor. A nonthermal process using acidified water was developed for producing anthocyanin-rich juice from PFSP and concurrently recovering native starch and dried pomace, which would increase the economic feasibility of the developed process. The results demonstrate an efficient process for the sweetpotato industry in producing PFSP pigmented juice and co-products for various food applications.

Introduction

Sweetpotatoes with purple-fleshed color are rich in beneficial phytochemicals such as phenolic acids and anthocyanins (Grace, Truong, Truong, Raskin, & Lila, 2015; Lee, Park, Choi, & Jung, 2013; Oki et al., 2003). This nutritious vegetable has been processed into various food products including beverages, natural colorants, and polyphenol-enriched functional food ingredients (Suda et al., 2003; Truong, Avula, Pecota, & Yencho, 2018). In addition to the color, many studies on nutraceutical foods have indicated that anthocyanins from purple-fleshed sweetpotatoes (PFSP) exhibited various potential health benefits including radical scav-

enging activity, anti-inflammatory activity, antimutagenic activity, reduction in memory impairment effects, reduction of body weight, and fat accumulation and liver injury in mice (Ju et al., 2017; Kano, Takayanagi, Harada, Makino, & Ishikawa, 2005; Suda et al., 2003; Wang, Nie, & Zhu, 2016; Wu et al., 2008; Zhang et al., 2009). An intake of 400 mg anthocyanins per day from PFSP juice showed a potential protection for the liver against oxidative stress in healthy men with borderline hepatitis (Suda et al., 2008). Protection against colorectal cancer as affected by anthocyanins from PFSP has been reported in studies with *in vitro cell culture* and *in vivo* animal model (Lim et al., 2013).

For juice and pigment extraction, sweetpotatoes are usually subjected to heat treatment for making into purees, which are the intermediate materials for subsequent processing operations (Truong et al., 2018). Heating the peeled sweetpotato slices at 90 °C for 10 min was reported for juice processing from PFSP (Suda et al., 2002). Blanching treatments at various temperatures (90 °C to 105 °C) and times (3 to 10 min) were reported by several investigators to inactivate the activities of polyphenol oxidase (PPO) and peroxidase associated with enzymatic darkening in processed slices of orange-fleshed sweetpotatoes (Ma, Silva, Hearnberger, & Garner, 1992) and anthocyanin degradation in juice extraction from PFSP (Cevallos-Casals & Cisneros-Zevallos, 2004). Preheating PFSP to a temperature range of 70 to 90 °C in water

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inactivated PPO and softened the tissue resulting in significant increase in polyphenolic extraction (de Aguiar-Cipriano, Ekici, Barnes, Gomes, & Talcott, 2015). Microwave baking PFSP roots for extraction of anthocyanins using acidified electrolyzed water was reported by Lu et al. (2010). However, heating gelatinizes starch and produces thick slurry with strong cooked flavor, which are drawbacks in PFSP juice and pigment extraction from this starchy vegetable. Tamaki, Tamaki, and Suzuki (2007) reported the use of activated carbon and maltosyl cyclodextrin to deodorize the off-odors of the saccharified substances formed during steam blanching of sweetpotatoes. Therefore, development of processes to overcome the stated problems would be beneficial to the sweetpotato processing industry.

Processing of raw PFSP into flours to be used in polyphenol extraction for further processing into functional products or analytical quantification has been reported in the literature (Ahmed, Akter, & Eun, 2011; Fan, Han, Gu, & Chen, 2008; Truong, Hu, Thompson, Yencho, & Pecota, 2012). However, converting raw sweetpotatoes into flours as intermediate material for juice processing increases energy consumption and production cost. An alternative approach to the stated blanching and flourintermediate process would be to extract juice and pigments directly from sweetpotatoes without heat treatment. This process can overcome the cooked-odor problem in the extracted juice and it allows the recovery of two co-products, raw starch and fiber residue. Inactivation of PPO and peroxidase in raw PFSP must be carried out by nonthermal methods. There are several alternatives to heat treatment for controlling enzymatic browning in fruits and vegetables, including high hydrostatic pressure, irradiation, pulse electric field and the use of chemicals, such as enzyme inhibitors, reducing agents, chelating agents, complexing agents, and acidulants (Queiroz, Mendes-Lopes, Fialho, & Valente-Mesquita, 2008). Among the acidulants, citric acid has been widely used in many foods including sweetpotato products (de Aguiar-Cipriano et al., 2015; Truong et al., 2010). Teangpook, Panthavee, Puminat &Thalang (2012) reported the use of 0.5% citric acid in blending raw PFSP for juice extraction and beverage formulations. However, the effects of acidification on inactivating PPO and peroxidase and anthocyanin recovery were not evaluated.

Therefore, this study aimed to: (1) determine the effects of acidification on PPO and peroxidase activities and pigment extraction from raw PFSP using acidified water as solvent, and (2) develop an integrated process for producing pasteurized anthocyanin-rich juice and concurrently recovering native starch and pomace as co-products for functional food applications.

Materials and Methods

Chemicals

Chlorogenic acid, Folin-Ciocalteau (FC) phenol reagent, catechol, and guaicol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate, sodium phosphate, sodium carbonate, and hydrogen peroxide (30%) were obtained from Fisher Scientific (Waltham, MA, USA). All chemicals used were analytical grade except citric acid that was the anhydrous food grade chemical from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Water used for HPLC analysis was purified with a deionized water system (Pure Water Solutions, Hillsborough, NC, USA).

Sweetpotato variety and juice extraction

Purple-fleshed sweetpotato variety (NC 413) was grown at the experimental fields of the Sweetpotato Breeding Program

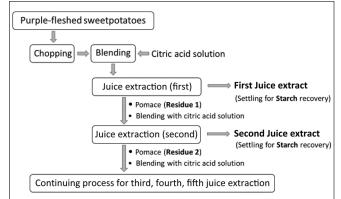


Figure 1–Flow diagram of juice extraction and recovery of co-products (starch and pomace as residues) from purple-fleshed sweetpotatoes (PFSP).

(Clinton, NC, USA), North Carolina State Univ. The harvested roots were cured at 30 °C, 85% to 90% relative humidity for 7 days, and stored at 14 °C, 85% to 90% relative humidity for about 2 to 3 months prior to sampling for this study. Two batches of NC 413 roots were used in the duplicated experiments. The roots were cut into 2.5 cm chunks and 150 g sample was blended with 150 mL of deionized water acidified with various citric acid concentrations (0% to 5% w/v) using a Waring blender (model 7009G, Waring Commercial, Torrington, CT, USA) for 2 min. After blending, the slurry was run through a juice extractor (Super Angel model 5500, U.S. Juicers, Tustin, CA, USA), which separated the juice containing raw starch from the fiber pomace, which was also referred as residue (Figure 1). The blender was rinsed with 20 mL of citric acid solution used during juicing. The final volume of the extracted juice was then brought to 250 mL with the same citric acid solution and aliquots were taken for measurements of pH, color values, and spectral scanning within 30 min at room temperature. Samples were also taken and stored in -80 °C freezer for enzyme assay and analysis of polyphenol content.

For the repeated extraction experiments, the solvent was 3% w/v citric acid solution. The first extraction was carried out as described above and the collected fiber pomace was subjected to four repeated extractions with 3% citric acid solution to individuate the number of extractions for extracting total phenolics (TP) and anthocyanins from PFSP. Aliquots of each extract were collected for analysis of polyphenol content.

Co-product recovery and proximate analysis

For co-product recovery, 2 kg of PFSP chunks were blended with 2 liters of 3% citric acid solution for 2 min using a heavyduty blender (model LBC 15, Waring Commercial, Torrington, CT, USA). The slurry was passed through the Super Angel juice extractor as described above. The extraction was repeated one more time and the extracted juices were placed in a 4 °C cold room overnight for raw starch settling. Juice from each extract was decanted and the starch was washed with deionized water as described by Walter, Truong, Wiesenborn, and Carvajal (2000). The recovered starch and fiber residues were dried in a convection oven at 50 °C for 10 hr. Samples of the dried fiber residue were analyzed for moisture, protein, fat, ash, and total dietary fibers by Microbac Laboratories Inc. (Pittsburg, PA, USA) following the AOAC Methods. Juice pasteurization

Extracted juice samples were packaged into aluminum foil pouches (Aseptia Inc., Raleigh, NC, USA) for pasteurization. Aliquots of juice samples (40 g) were weighed into pouches and sealed above and below the closure seam using an electric sealer (American International Electric Impulse Sealer, Industry, CA, USA). The aluminum foil pouches were submerged in an oil bath circulator (Model RTE-111, NESLAB Instruments Inc., Portsmouth, NH, USA) for juice pasteurization at 60 °C, 65 °C, 70 °C, 75 °C, and 80 °C for 762, 264, 90, 30, and 12 s, respectively, as recommended for pasteurization of acidified food products (Breidt, Sandeep, & Arritt, 2010). Processing times were recorded after the come-up time of 3 min, the time needed to get from the juice temperature of 22 °C to the process temperature. Temperatures were recorded using a thermocouple interfaced with a digital thermometer (Model HH 502, Omega Inc., Stamford, CT, USA). The processed pouches were immediately placed in an ice water bath and samples were taken for determining the polyphenol retention. All samples were processed in duplicate.

Enzyme assays

PPO and peroxidase activities were assayed at room temperature (22 °C) by measuring the initial rate of increase in absorbance using a Varian spectrophotometer, Cary WinUV model 300 (Palo Alto, CA, USA). For each assay, PFSP juice or enzyme extract was added to the substrate solution. The cuvette was inverted and increase in absorbance was immediately measured. Catechol was used as the substrate for PPO assay and absorbance was measured at 420 nm (Ma et al., 1992). A 3 mL of reaction mixture contained 0.3 mL of PFSP juice or enzyme extract and 2.7 mL of 0.1 M catechol in 0.2 M sodium phosphate buffer (pH 6.0). For peroxidase activities, 100 µL of PFSP juice or enzyme extract was added to 2.9 mL of 100 mM citrate-phosphate buffer (pH 5.5), containing 18.2 mM guaiacol and 4.4 mM H_2O_2 as substrates, and the absorbance change at 470 nm was measured (Castillo Leon et al., 2002). One unit of PPO or peroxidase activity is defined as the amount of enzyme that causes an increase in absorbance (ΔA) of 0.01/min.

Quantitation of total phenolics and total monomeric anthocyanins

TP were quantitated using a modified FC method as described by Steed and Truong (2008). Chlorogenic acid was used as standard. Sample or standard solutions (0.25 mL) were diluted with deionized water (3.75 mL) to which 0.5 mL of the FC reagent was added and the mixture was allowed to react at room temperature for 3 min. Sodium carbonate (1 N, 0.5 mL) was added and the reaction was carried out for 1 hr. The absorbances of all solutions were read at 725 nm using a Varian spectrophotometer (Cary WinUV model 300). A blank that contained 0.25 mL of water instead of sample, along with the same amount of water for dilution, FC reagent, and sodium carbonate solution was used to obtain the absorbance for a baseline. TP values were reported in milligrams of chlorogenic acid equivalents per 100 g fresh weight of PFSP or liter of juice.

Total monomeric anthocyanin (TMA) content was determined following the pH-differential method as described by Giusti and Wrolstad (2001). Two dilutions were performed on each sample and the absorbance readings at 530 nm were less than 1.2. The first dilution used potassium chloride (0.025 M) at pH 1.0 and the second was with sodium acetate (0.4 M) at pH 4.5. All diluted solutions were allowed to equilibrate for 15 min before absorbance at 530 and 700 nm were measured using a spectropho-

tometer calibrated with deionized water as the blank. The difference in absorbance between the two pH values and wavelengths was used to calculate anthocyanin content as cyanidin-3-glucoside with molecular weight of 449.2 g/mol and molar absorptivity of 26,900 L/cm/mol. The TMA content was reported as milligrams anthocyanins per 100 g fresh weight of PFSP or liter of juice.

Color and pH measurements

The pH values of extracted juices were measured with an Accumet AR 50 pH meter (Fisher Scientific). Color of the extracted juices was measured spectrophotometrically using a Varian spectrophotometer, Cary WinUV model 300. All measurements were performed at least in duplicate. Spectral characteristics of the extracts were scanned at 380 to 780 nm. The color values were expressed as tristimulus parameters (L^* , a^* and b^* , CIELAB system) and calculated using a color application software (Varian Australia Pty. Ltd., Victoria, Australia). L^* (lightness, 0 for black, 100 for white), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* (-b = blueness, +b = yellowness). Hue angle indicates sample color (0° or 360° = red; 90° = yellow; 180° = green; 270° = blue), and chroma indicates color purity or saturation. Hue angle (H° , Eq. 1 and 2), chroma (C^* , Eq. 3) were calculated using the following equations:

$$H^{\circ} = \tan^{-1} (b^*/a^*)$$
 when $a^* > 0$ and $b^* \ge 0$ (1)

$$H^{\circ} = 360^{\circ} + \tan^{-1} (b^*/a^*)$$
 when $a^* > 0$ and $b^* < 0$ (2)

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$
(3)

Statistical analysis

The experiment was conducted with two replicates in a randomized complete block design. Two samples were taken and analyzed per replicate. 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 using SAS Statistical Analysis System, v. 9.1 (SAS Inst. Inc., Cary, NC, USA).

Results and Discussion

Effects of acidification on enzyme activities and polyphenolic extraction

As shown in Table 1, an increase in citric acid concentration in the PFSP blends resulted in lowering pH of the extracts and consequently a decrease in both PPO and peroxidase activities. PPO was inactivated at 1.0% w/v citric acid (pH = 3.87) while higher citric acid level up to 4% (pH = 2.80) was required to effectively inactivate peroxidase activities. About 80% of the peroxidase activity in the water extract (pH = 6.02) was inactivated by decreasing pH to 3.02 with 3% citric acid and the enzyme activity was not detected in the juice extracted with 4% citric acid (pH = 2.8). The results are in agreement with previous reports on low activities below the optimum pH range of 3.5 and 5.5 for sweetpotato peroxidase that was assayed with various substrates including guiacol in this study and chlorogenic acid, the main phenolic acids in sweetpotatoes (Castillo Leon et al., 2002; Truong, McFeeters, Thompson, Dean, & Shofran, 2007). For sweetpotato PPO activity, the optimum pH also varies with the substrates, from about 4.0 for chlorogenic acid

Table 1-Acidification effect on polyphenol oxidase (PPO) and peroxidase activities in PFSP juice.

Citric acid (% w/v)	Extract pH	PPO activity units	Peroxidase activity units
0	6.02	3350.8 ± 117.7	1139.4 ± 4.2
0.25	4.84	431.7 ± 69.9	984 ± 76.6
0.5	4.34	5.0 ± 1.5	1014.1 ± 33.4
0.75	4.1	8.3 ± 4.9	747.3 ± 30.9
1	3.87	nd	796.0 ± 20.0
1.5	3.6	nd	773.3 ± 8.2
2	3.35	nd	523.8 ± 14.0
3	3.02	nd	229.2 ± 23.5
4	2.8	nd	nd
5	2.8	nd	nd

Values are means of two replicates, nd = not detected.

to 6.5 for catechol, which was the substrate used in this study. Unlike peroxidase, the activities of PPO were significantly reduced (P < 0.01) at the lowest citric acid concentration of 0.25%, pH = 4.84 and not detectable at citric acid concentration of $\geq 1\%$ (pH \leq 3.86). Lourenco, Neves, and Da Silva (1992) reported the optimum pH of 4.5 for sweetpotato PPO activities with chlorogenic acid as the substrate. Therefore, the inactivation of sweetpotato peroxidase and PPO can be simply achieved by lowering the pH with food grade acids during juice extraction. In addition to decreasing pH, organic acids, such as citric acid, can chelate the metal cofactors, denature the protein structure of PPO and peroxidase enzymes and consequently prevent the enzymatic browning oxidation of phenolic compounds in fruits and vegetables (Kader, Irmouli, Nicolas, & Metche, 2002; Yoruk & Marshall, 2005). As compared to heating, Chhe, Imaizumi, Tanaka, and Uchino (2018) recommended blanching temperature of higher than 94 °C for inactivating peroxidase activity in sweetpotato slices. Ma et al. (1992) also reported water blanching at 94 °C for 5 min or at 100 °C for 3 min for inactivating PPO and minimizing enzymatic darkening in processed slices of orange-fleshed sweetpotato cultivars. Steam blanching of red sweetpotato slices at 105 °C for 2 and 10 min can inactivate the activities of anthocyanin-degrading enzymes, especially peroxidase by 99% and 100%, respectively (Cevallos-Casals & Cisneros-Zevallos, 2004). Tissue softening and PPO inactivation during preheating of PFSP (70 °C to 90 °C) significantly increased the extraction and recovery of anthocyanins and phenolic acids suitable for food uses (de Aguiar-Cipriano et al., 2015). However, recovery of raw starch and cell wall residues from sweetpotatoes roots would not be feasible in the process involving a preheating step. Starch gelatinization in thermally treated sweetpotato roots results in thick slurry that can be a drawback in the processing operation for juice or pigment extraction. Furthermore, the cooked flavor of sweetpotatoes may not be suitable for uses in some formulated food products.

Visible absorption spectra (380 to 700 nm) of PFSP extracts with different citric acid concentrations are shown in Figure 2. All the extracts exhibited maximal absorption wavelength (λ_{max}) at 530 nm that has been previously reported for analyzing the TMA content in various PFSP varieties (Truong et al., 2012). Absorbance at λ_{max} and intensity of reddish color of the extracts increased with citric acid concentrations (Figure 2). The decrease in pH associated with citric acid addition reduced enzymatic degradation of polyphenols and intensified the red and blue colors of the extracted anthocyanin components from PFSP, peonidin, and cyanidin (Truong et al., 2010), as indicated by the color values

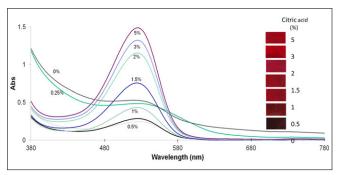


Figure 2–UV-visible spectral characteristics and color of PFSP extracts at different citric acid concentrations (% w/v).

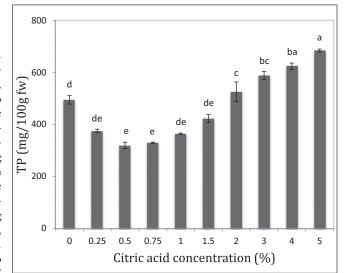


Figure 3–Effect of acidification on extraction of total phenolics (mean values having different letters are significantly different at P < 0.05).

of the juice in Table 2. There were no significant effects of citric acid concentrations at 3% to 5% (pH < 3.0) on all color values $(L^*, a^*, b^*, hue, and chroma)$ of the extracted PFSP juice. These PFSP extracts are characterized by average values of lightness (L^* = 67.24), red color ($a^* = 67.11$), blue color ($b^* = -0.52$), hue angle ($H^{\circ} = 359.55$), and vivid color ($C^* = 67.11$) located in the purple-red color region of the CIELAB space. The acid treatments inactivated POP and PO activities resulting in significant increases in both TMA and TP from PFSP (Figures 3 and 4). The TP values increased from 87.2 to 477.8 mg/100g fw, and TMA from 8.56 to 87.21 mg/100g fw when citric acid concentration increased from 0% (pH = 6.0) to 3% (pH = 3.0). The lowest TP levels of the PFSP extracts at 0.25% to 0.75% citric acid (pH = 4.8 to 4.10) shown in Figure 3 could be partially explained by the low solubility of protein-polyphenol conjugate at the isoelectric point region of pH 4.0 of sweetpotato proteins (Mu, Tan, & Xue, 2009). The purple sweetpotato juices extracted with 0.5% citric acid as described by Teangpook, Panthavee, Puminat, and Thalang (2012) for uses in beverages would have low TP and TMA. The polyphenolic compounds in the extracts could interact with the soluble proteins to form protein-polyphenol compounds, which are less soluble at those citric acid concentrations. As the pH shifted away from the isoelectric point, the increase in solubility of the protein-polyphenol conjugates in 1% to 2% citric acid solutions could contribute to the increasing TP and TMA concentrations in the extracts. Further increase in citric acid concentration (3%

Table 2-Color values of PFSP juice as affected by citric acid concentration.

Citric acid (% w/v)	L^*	a*	b^*	Hue	Chroma
0	$71.32 \pm 0.72 \text{ d}$	$21.56 \pm 0.52 \mathrm{f}$	17.80 ± 0.39 b	$39.55 \pm 0.07 \text{ f}$	27.96 ± 0.65 e
0.25	$69.96 \pm 0.67 d$	$19.34 \pm 0.43 \text{ g}$	22.69 ± 0.39 a	49.55 ± 0.14 e	$29.81 \pm 0.58 e$
0.50	$88.57 \pm 0.28 a$	18.62 ± 0.41 g	$4.10 \pm 0.10 \text{ c}$	$12.42 \pm 0.02 \text{ g}$	$19.07 \pm 0.42 \text{ g}$
0.75	88.06 ± 0.34 a	$23.90 \pm 0.61 e$	$-0.71 \pm 0.02 \text{ d}$	$358.30 \pm 0.00 \mathrm{b}$	$23.91 \pm 0.61 \mathrm{f}$
1.0	84.33 ± 0.06 b	$34.24 \pm 0.10 \text{ d}$	$-4.47 \pm 0.01 \text{ e}$	$352.56 \pm 0.04 d$	$34.53 \pm 0.10 \text{ d}$
1.5	77.17 ± 0.19 c	$51.22 \pm 0.37 \text{ c}$	$-6.36 \pm 0.01 \text{ f}$	$352.92 \pm 0.06 d$	$51.61 \pm 0.37 c$
2.0	$70.68 \pm 0.04 \mathrm{d}$	$62.11 \pm 0.08 \text{ b}$	$-3.33 \pm 0.02 \text{ e}$	$356.93 \pm 0.03 c$	$62.20 \pm 0.08 \text{ b}$
3.0	67.88 ± 1.10 e	66.13 ± 1.38 a	$-0.97 \pm 1.08 \text{ d}$	359.15 ± 0.96 ab	66.14 ± 1.37 a
4.0	67.14 ± 0.11 e	$67.50 \pm 0.16 a$	$-0.39 \pm 0.12 \text{ d}$	$359.67 \pm 0.10 a$	67.50 ± 0.16 a
5.0	$66.69 \pm 0.12 e$	$67.70 \pm 0.17 a$	$-0.19 \pm 0.12 \text{ d}$	$359.84 \pm 0.11 a$	$67.70~\pm~0.17~{\rm a}$

Values within columns having different letter are significantly different (P < 0.05).

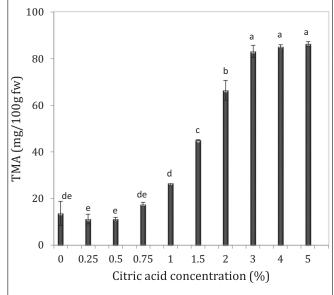


Figure 4–Effect of acidification on extraction of anthocyanins (mean values having different letters are significantly different at P < 0.05).

to 5%) had no significant (P > 0.05) increases in the extracted TP and TMA levels (Figure 3 and 4). The TMA content of PFSP corresponding to 3% to 5% citric acid solutions were at 80.41 to 87.20 mg/100 g fw, which were comparable to the values reported for purple-fleshed sweetpotato cultivars in previous studies and other commodities namely grapes (27 to 120 mg/100 g fw), plum (19 to 124 mg/100 g fw), sweetcherries (122 mg/100 g fw), and raspberries (92 mg/100 g fw) (Truong et al., 2010). Therefore, acidified water was effective in controlling polyphenolic degrading enzymes in PFSP resulting in attractive reddish color of anthocyanin-rich extracts.

Repeated extraction and recovery of co-products

Based on the above results, extraction using 3% citric acid solution was conducted for determining the number of repeated extractions (Figure 1) required to obtain the highest amount of phenolics and anthocyanins from PFSP. Table 3 shows that the yields of 82% TP and 49% TMA were obtained from the first extraction. About 93% TP and 83% TMA in PFSP were extracted by the first and second extraction. However, the third extraction contributed less than 4% and 16% to the total extracted TP and TMA. Combining all three extractions, the yields of TP were up to 97% and TMA to 99%. Apparently, the fourth and fifth ex-

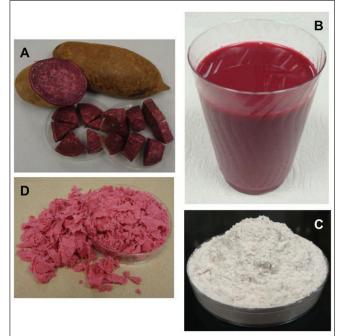


Figure 5–Raw PFSP (A), juice (B), starch (C), and high dietary fiber pomace (D).

traction appeared unnecessary for juice processing of PFSP. The combined PFSP juices from the first and second extractions had high yields of TP (1,850 mg/L) and TMA (475 mg/L), which are comparable with a preheating process for anthocyanin extraction from a PFSP variety reported by de Aguiar-Cipriano et al. (2015). The TP and TMA concentrations in the extracted juices in this study were comparable with those of the juices from various polyphenol-rich fruits such as blueberries, blackcurrant, and muscadine grape (Grace et al., 2015). Pasteurization of the PFSP juices at pH 3.0 in oil bath up to 75 °C to 80 °C for 12 to 30 s as recommended for thermal processing of acidified foods (Breidt et al., 2010) resulted in 14.7% loss in TMA and had no effect on TP (Table 4). Li et al. (2013) reported that anthocyanin compounds from PFSP are more stable during thermal processing than those from blackcurrant, blackberry, and other fruits. The thermal stability could be due to the acylation of most anthocyanins in PFSP (Truong et al., 2010).

An advantage of juice extraction by acidified water without heat treatment would be the recovery of co-products. The blended juice was passed through an extractor for separating the juice from

Table 3-Repeated extraction and yields of total anthocyanins and total phenolics.

Sequence of extraction	Total phenolics in juice (mg/L juice)	Total phenolics extracted from PFSP (mg/100 g roots)	Percent of total	Anthocyanins in juice (mg/L juice)	Anthocyanins extracted from PFSP (mg/100 g roots)	Percent of total
First	3193.12 ± 166.73	532.19 ± 27.79	81.62	566.40 ± 50.63	94.40 ± 8.44	49.38
Second	507.55 ± 124.92	84.59 ± 20.82	12.97	383.45 ± 63.91	63.91 ± 10.65	33.43
Third	149.00 ± 35.08	24.84 ± 5.85	3.81	189.93 ± 30.43	31.65 ± 5.07	16.56
Fourth	45.64 ± 18.33	7.61 ± 3.05	1.17	5.40 ± 0.83	0.90 ± 0.14	0.47
Fifth	16.75 ± 8.26	2.80 ± 1.38	0.43	1.75 ± 0.38	0.30 ± 0.06	0.15
Total	3912.06 ± 353.33	652.02 ± 58.89	100	1146.94 ± 146.18	191.16 ± 24.37	100

Table 4-Effect of pasteurization on total phenolics and anthocyanins in the extracted PFSP juices.

Temperature (°C)	Time(s)	Total phenolics (mg/100 mL juice)	Total monomeric anthocyanins (mg/100 mL juice)
60	762	205.98 ± 17.62	33.55 ± 5.64
65	264	244.19 ± 15.61	28.60 ± 15.54
70	90	260.04 ± 10.28	30.12 ± 6.72
75	30	221.28 ± 19.65	27.74 ± 1.16
80	12	264.37 ± 26.37	27.86 ± 1.04

Values are the means of three replicates.

Table 5-Co-products recovered from the first and second extraction.

Products	First juice extraction	Second juice extraction	Total
Dried starch [*] (g/kg fresh roots)	140.92 ± 2.04	26.62 ± 7.08	167.54 ± 5.06
Dried pomace ^{**} (g/kg fresh roots)	83.38 ± 11.42	56.08 ± 0.89	139.46 ± 10.53

Oven dried at 50 °C for 3 hr, cooled to room temperature.

*Starch: vacuum filtered, washed with thin layer of 95% ethanol, air dried for 3 days.

**Residue 1 & 2: oven dried at 50 °C for 8 hr, cooled to room temperature.

the pomace. The collected juice contained all soluble substance including TP, TMA, and also nonsoluble particles especially raw starch granules, which could be obtained by centrifugation or settled during overnight storage in a cold room (Walter et al., 2000). Images of extracted juice, dried starch, and dried pomace obtained from PFSP roots are shown in Figure 5. Based on the results of $2 \times$ extractions from 1 kg of fresh PFSP, about 3.2 liters of juice containing 6.20 g TP and 1.58 g TMA can be obtained together with 167 g dried starch and 140 g dried residue as the co-products (Table 5). Therefore, it can be estimated that 17% starch and 14% residue can be recovered from raw PFSP as co-products contributing to the increase in the economic feasibility of the developed process. These yield values would be varied with the PFSP varieties. The physiochemical properties of the starch recovered from this acidified extraction of PFSP needs to be evaluated for applications as an ingredient in various processed foods and industrial products. In Asia, sweetpotato starch has been produced by small and medium-scale factories for utilization in processing of noodles, thickening agents, syrups, fermented products, and various chemicals (Truong et al., 2018).

Proximate composition, including moisture, protein, fat, ash, total dietary fiber, total carbohydrate, and calories of the samples from dried PFSP, residues from the first and second extraction are presented in Table 6. The nutrient contents of dried PFSP were within the ranges of crude protein (4.07% to 6.20%) and ash (2.33% to 4.48%) but lower in fat (1.27% to 2.16%) and carbohydrate (81.3% to 87.3%) as compared to the values reported for the common sweetpotato varieties grown in the United States (Cartier et al., 2017; Truong et al., 2018). It is known that agricultural practices and environment factors can have effects on the

Table	6-Chemical	composition	of	raw	PFSP	and	pomace
(residu	es 1 and 2).	-					-

Composition	Dried PFSP Roots	Residue 1 (g/100g dried powder)	Residue 2
Moisture	5.31 ± 0.01	4.53 ± 0.88	4.41 ± 0.62
Protein	5.79 ± 0.01	4.17 ± 0.11	3.49 ± 0.53
Fat	0.91 ± 0.16	0.45 ± 0.08	0.55 ± 0.16
Ash	4.03 ± 0.04	1.45 ± 0.25	0.62 ± 0.04
Total dietary fiber	8.60 ± 0.42	25.55 ± 1.06	36.15 ± 2.90
Carbohydrate	75.56 ± 0.28	63.86 ± 0.46	54.80 ± 2.86
Calories	390.38 ± 0.93	395.03 ± 2.55	396.61 ± 5.50

growth and nutrient accumulation in fruits and vegetables. As anticipated, the juicing process extracted TP and TMA, as well as other nutrients from PFSP. Protein, fat, ash, and carbohydrate contents in the residues of the second extraction were reduced, respectively, by 44, 40, 85, and 28% of the levels in the dried raw PFSP (Table 6). However, the dietary fiber was mostly retained with percentage of 25.55 g/100 g and 36.15 g/100 g in the residue 1 and residue 2, which were respectively about 2.9 and 4.2 times higher than the total dietary fiber value in PFSP. Therefore, the residues of the juice extraction form PFSP could be considered as the high dietary fiber ingredient that can potentially be used in various formulations of health food products and other uses.

Conclusions

Acidified water was effective in controlling polyphenolic degrading enzymes in PFSP resulting in attractive reddish color of anthocyanin-rich extracts. Over 80% TMA and 93% TP in

PFSP were extracted by the first and second extraction. About 17% starch and 14% residue can be recovered from raw PFSP as co-products, which would increase the economic feasibility of the developed process. With high polyphenolic content, the PFSP juice can be used as functional ingredient in beverages and nutraceutical products.

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Authors' Contributions

ANT conducted the experiments, analyzed the data, interpreted the results, and drafted the manuscript. YT performed the experiment on juice extraction and pasteurization. GKH collaborated in the analysis of polyphenolics and revised the manuscript. JS participated in designing the study and revised the manuscript. VDT conceptualized the study, supervised the execution of the experiments, assisted in interpretation of the results, reviewed, and revised the manuscript.

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