Industrial Crops and Products 32 (2010) 613–620

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Extraction of anthocyanins from industrial purple-fleshed sweetpotatoes and enzymatic hydrolysis of residues for fermentable sugars

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ARTICLE INFO

Article history:
Received 20 May 2010
Received in revised form 19 July 2010
Accepted 21 July 2010

Keywords:
Purple-fleshed sweetpotato
Solvent extraction
Anthocyanins
Liquefaction
Saccharification
Ethanol

ABSTRACT

Recent trends in health and wellness as well as fossil fuel dependent markets provide opportunities for agricultural crops as renewable resources in partial replacement of synthetic components in food, clothing and fuels. This investigation focused on purple-fleshed industrial sweetpotatoes (ISPs), a crop which is used for industrial purposes because it produces relatively high quantities of antioxidants in the form of anthocyanins as well as high starch content for potential hydrolysis into fermentable sugars. Laboratory extraction and enzymatic hydrolysis studies were conducted on purple-fleshed ISPs in order to evaluate the effects of solvent, extraction temperature and solid loading on recovery of anthocyanins and fermentable sugars. Total monomeric anthocyanin and phenolic concentrations of the extracts were measured. Residual solids from anthocyanin extraction were subsequently hydrolyzed for sugar production (maltotriose, maltose, glucose and fructose). Extraction temperature of 80 °C using acidified methanol at 3.3% (w/v) solid loading showed the highest anthocyanin recovery at 186.1 mg cyanidin-3-glucoside/100 g fw. Acidified solvents resulted in 10–45% and 16–46% more anthocyanins than non-acidified solvents of ethanol and methanol, respectively. On average, glucose production ranged from 268 to 395 mg/g dry ISP. Solid residues that went through extraction with acidified ethanol at 50 °C at 17% (w/v) solid loading had the highest average production of glucose at 395 mg/g dry ISP. Residues from methanol solvents had lower glucose production after hydrolysis compared to those of ethanol based extraction. Fermentation of produced sugars from ISP residues was limited, where 38% less ethanol was produced from extraction residues compared to treatments that did not undergo initial extraction. Overall, purple-fleshed ISPs are amenable to anthocyanin and phenolic extraction, making it a suitable substrate for development of industrial colorants and dyes. However, more research is needed to obtain a suitable extraction point when trying to achieve a high recovery of anthocyanins and effective starch conversion to fermentable glucose.

1. Introduction

Anthocyanin pigments are responsible for the red, purple and blue colors of many fruits, vegetables, cereal grains and flowers. They are members of a class of water soluble, terrestrial plant pigments that are classified as phenolic compounds collectively named flavonoids. These pigments can exist in many different structural forms and related physico-chemical phenomena have a profound effect on their actual color and stability (Delgado-Vargas and Paredes-Lopez, 2003).

Interest in anthocyanin pigments in the consumer market has increased recently due to their potential health benefits as dietary antioxidants and the range of colors they produce with potential as a natural dye. Anthocyanins are characterized as having an electron deficiency due to their particular chemical structure, which makes them very reactive toward free radicals present in the body, consequently enabling them to be powerful natural antioxidants (Galvano, 2005). Anthocyanins in foods also provide advantages in anti-cancer, liver protection, reduction of coronary heart disease and improved visual acuity applications (Timberlake and Henry, 1988; Francis, 1989; Mazza and Miniati, 1993; Bridle and Timberlake, 1996). In addition, the deep purple–red color of anthocyanins makes them an attractive source of natural food colorant for the food and textile industry as an alternative to synthetic food dyes (Wegener et al., 2009).

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doi:10.1016/j.indcrop.2010.07.020

Table 1
Anthocyanin content of some common fruits and vegetables.

Source	Pigment content (mg/100 g fresh weight)
Plum[1]	2–25
Red onions[2]	7–21
Red radishes[3]	11–60
Strawberries[1]	15–35
Red raspberries[2]	20–60
Red cabbage[1]	25
Blueberries[2]	25–495
Blackberries[2]	83–326
Cranberries[1]	60–200
Grapes[2]	6–600
Purple-fleshed sweetpotatoes[4]	84–174

[1]Timberlake (1988), [2]Mazza and Miniati (1993), [3]Giusti et al. (1998) and [4]Steed and Truong (2008).

Purple-fleshed ISPs (*Ipomoea batatas*) accumulate large amounts of anthocyanins in the storage roots. In comparison to other common anthocyanin containing fruits and vegetables, the concentration of anthocyanins in purple-fleshed ISPs are in the same range as some of the highest anthocyanin producing crops like blueberries, blackberries, cranberries and grapes (Table 1). Purple-fleshed ISP anthocyanins exist in mono- or diacylated forms of cyanidin and peonidin and have been regarded as a source of food colorant with high colorant power and stability (Odake et al., 1992; Goda et al., 1997; Philpott et al., 2003; Terahara et al., 2004). These forms of anthocyanins also contribute to a high antioxidant activity for purple-fleshed ISPs compared to sweetpotatoes of white, yellow and orange flesh colors (Teow et al., 2007).

Isolation of anthocyanin pigments from plants is typically done using solvent extraction processes (Kong et al., 2003). Anthocyanins are polar molecules and consequently more soluble in polar solvents, however extraction conditions are also key factors in their overall solubility (Delgado-Vargas and Paredes-Lopez, 2003; Kong et al., 2003). Research on extracting anthocyanins from fruits and vegetables including purple-fleshed sweetpotato powder, purple corn, red and black currants, and grapes have shown that alcoholic extraction is suitable. The extraction conditions such as solid–liquid ratio (solid loading), incubation temperature, incubation time, solvent type and solvent concentration are important in the stability and concentration of anthocyanins that can be extracted from these particular crops (Oki et al., 2002; Pascual-Teresa et al., 2002; Lapornik et al., 2005; Jing and Giusti, 2007; Fan et al., 2008; Steed and Truong, 2008). Methanol is the most commonly used solvent, but it is also considered more toxic and hazardous to handle than other alcohols. Ethanol for example is more environmentally friendly and can also recover anthocyanins with good quality characteristics (Delgado-Vargas and Paredes-Lopez, 2003). These studies on anthocyanin extraction have been limited to the use of one combination of solvent, solid loading and incubation temperature.

Purple-fleshed ISPs are different from standard table-stock sweetpotatoes in the U.S. in that they have been bred not only for higher anthocyanin content, but also higher dry matter content (~32% dry matter on average) in the form of starch. The high dry matter can be converted enzymatically by a process called hydrolysis into simple sugars (e.g. glucose), making these sweetpotatoes a potential candidate as a feedstock for bioethanol and biobased product production (Nichols, 2007). To date, limited research has been conducted on purple-fleshed ISPs to examine the effect of anthocyanin extraction on the sugar production potential from the solid residue during a subsequent hydrolysis and ethanol fermentation process.

Experiments were performed to evaluate the effects of solvent type, solid loading, and incubation temperature on total monomeric anthocyanin and phenolic concentrations during anthocyanin extraction from purple-fleshed ISPs. In addition, the effect of initial extraction conditions on the production of fermentable sugars from purple-fleshed ISP starch during a subsequent hydrolysis process was examined.

2. Materials and methods

2.1. Extraction solvents, commercial enzymes and yeast culture

Methanol (A45204, Fisher Scientific) and glacial acetic acid (A35-500, Fisher Scientific) were of HPLC analytical grade, ethanol (Cat# E190, Pharmco-AAPER) was of USP grade.

Alpha amylase randomly cleaves the inner portions of amylose (α-1,4 bonds) to form soluble dextrins. The α-amylase used was Liquozyme SC (Novozymes, North America, stored at 4 °C, density 1.25 g/ml) with an optimal pH 5.5, optimal temperature of 85 °C and activity of 120 KNU-S/g enzyme. A kilo novo unit, KNU-S, is the amount of enzyme that breaks down 5.26 g of starch per hour. Glucoamylase cleaves the α-1,4 links, releasing glucose molecules from the non-reducing end of the amylose chain, and also acts on the α-1,6 branch links, which are hydrolyzed but less rapidly (Heldt and Heldt, 2005; Roy and Gupta, 2004). The glucoamylase used was Spirizyme Ultra (Novozymes, North America, stored at 4 °C, density 1.15 g/ml) with an optimal temperature of 65 °C and activity of 900 AGU/g protein. An amyloglucosidase unit, AGU, is the amount of enzyme able to hydrolyze 1 μmol of maltose per minute at 37 °C and a pH of 4.3.

Ethanol Red Yeast (Lesaffre Yeast Corp., Milwaukee, WI) was used in all ISP fermentations at a dry weight concentration of 0.1% (w/v). Yeast cell concentrations were on average 5.6×10^7 cells/ml once rehydrated.

2.2. Industrial sweetpotato preparation

The purple-fleshed ISP line NC-413 was used for all experiments. All materials were grown and harvested during the 2008 cropping season at the Cunningham Research Station (Kinston, NC, F1 Field, Latitude 35.2977, Longitude 77.5754). After harvest, the storage roots of NC-413 were cured (85 °F, 85% rh, 7 days) and transferred to long-term storage (58 °F, 85% rh, 8 months). Roots of purple-fleshed ISPs were washed and dried (58 °F, 2 days).

2.3. Experimental design and statistical analysis

The effects of solvent (70% ethanol, 70% acidified ethanol, 70% methanol and 70% acidified methanol), extraction temperature (25, 50, 80 °C) and solid loading (3.3%, w/v, 17%, w ISP/v solvent) on total monomeric anthocyanin and phenolic concentrations resulting from extraction of purple-fleshed sweetpotatoes were investigated. All treatment combinations in this $4 \times 3 \times 2$ full factorial experimental design were completed in triplicate with duplicate control combinations (sterile water instead of solvent). Residual solids from the described extraction treatment combinations were carried forward to examine the effects of the extraction conditions on sugar production and starch degradation during subsequent hydrolysis. All extraction/hydrolysis treatment combinations were completed in triplicate with duplicate control combinations (no extraction with hydrolysis enzymes). Response variables for this experiment included total monomeric anthocyanin and phenolic concentration after extraction as well as sugar production and change in starch content after hydrolysis of residual extraction solids.

In a secondary experiment, fermentability of sugars produced from extraction residues was further examined by selecting three extraction conditions (70% acidified ethanol at 50 °C, 70% acidified

ethanol at 80 °C and 70% acidified methanol at 80 °C) and completing hydrolysis of purple-fleshed ISP solids at two enzyme loadings (2.5, 5.0 AGU/g dry ISP) to generate sugar feedstocks for use in ethanol fermentation.

Analysis of variance for main and interaction effects and *t*-test comparisons were evaluated using PROC GLM in SAS 9.1 software (SAS® Inc., Cary, NC) for the factorial experiment studying the effects of extraction treatment combinations on response variables key to the extraction and hydrolysis processes. Assessment of statistical significance was made at an α value of 0.05.

2.4. Extraction of anthocyanins and subsequent hydrolysis of ISP residues

ISP roots were sliced (transverse direction, 2–3 mm thickness chips) and diced (food chopper, ~3 mm^3). Diced roots (5.15 g fresh ISP (70.9% $MC_{wet\text{-}basis}$, 1 dry g ISP)) were measured into sterile 50 ml conical Falcon tubes. Solvents (70% ethanol (pH ~ 5.5), acidified ethanol (pH ~ 3.5)—70% ethanol with 7% acetic acid, 70% methanol (pH ~ 5.5), acidified methanol (pH ~ 3.5)–70% methanol with 7% acetic acid) were added to treatment tubes and sterile water was added to controls, both at 3.3% (w/v) and 17% (w/v) solid loadings. All tubes (except controls not undergoing extraction) were shaken (80 rpm) and incubated for 1 h in a water bath at the appropriate temperature level (25, 50 or 80 °C). Tubes were centrifuged (15 min, 2731 × *g*, 4 °C) and a portion of the supernatant (2 ml) was removed and stored at −80 °C until anthocyanin and phenolic analysis. All samples were analysed within a week.

The residual solid portion was washed with deionized distilled water (12 ml, discarding supernatant each time), vortexed and centrifuged (15 min, 2731 × *g*, 4 °C). The washing process was repeated twice. Sodium azide (0.2%, w/v) was added to washed solids and controls as a preservative. The volume in all tubes was adjusted to 12.5% (w/v) (g dry ISP/ml solution) with sterile water and the pH was adjusted to 5.5 with 2 M NaOH (20–30 μl). Liquozyme SC was added to all tubes at a level of 0.30% volume of enzyme/g dry ISP (4.5 KNU-S/g dry ISP). Treatments were shaken (80 rpm) and incubated for 2 h in a water bath at 85 °C. Spirizyme Ultra (5.0 AGU/g ISP solid) was added to all tubes and were incubated at 65 °C in a shaking (80 rpm) water bath for 24 h. Initial sugar content was sampled at time 0. Final sugar content was measured after saccharification where tubes were centrifuged (15 min, 2731 × *g*, 4 °C) and a portion of the supernatant (2 ml) was removed and stored at −80 °C until HPLC sugar analysis. The remaining supernatant after saccharification was discarded, the residual solids washed with deionized distilled water (12 ml), vortexed and centrifuged (15 min, 2731 × *g*, 4 °C). The washing process was repeated twice and solid portions were stored in a −20 °C freezer (up to 3 days) prior to analyse for alcohol insoluble solids (AIS).

2.5. Starch conversion and ethanol production from ISP extraction residues

Diced roots (16.08 g fresh ISP (68.9% $MC_{wet\text{-}basis}$, 5 dry g ISP)) were measured into sterile 50 ml conical Falcon tubes. Solvents (acidified ethanol and acidified methanol) were added to treatment tubes and sterile water was added to controls at 17% (w/v) solid loading. All tubes (except controls not undergoing extraction) were shaken (80 rpm), and incubated for 1 h in a water bath at temperatures of either 50 °C (acidified ethanol) or 80 °C (acidified ethanol and acidified methanol). Centrifugation, washing, and liquefaction were performed as described previously with the washing process repeated three times in this experiment. Spirizyme Ultra was randomly added to select tubes at 2.5 and 5.0 AGU/g ISP solid to create triplicate treatment combinations with the three extraction conditions and the controls that went through hydrolysis only. Samples were taken at time zero of liquefaction to estimate initial sugar content. After hydrolysis tubes were centrifuged (15 min, 2731 × *g*, 4 °C) a portion of the supernatant (2 ml) was removed and stored at −80 °C until sugar analysis for final sugar content. The remaining supernatant/hydrolysate was saved for fermentation. Culture tubes (25 ml) with purple ISP hydrolysate (10 ml) from the different extraction–hydrolysis combinations were autoclaved (15 min, 121 °C, 15 psi). Yeast (0.1%, w Ethanol Red®/v) was added to purple ISP sugars in the culture tubes after cooling and cultures were incubated in a water bath at 37 °C for 120 h. Treatment fermentations were completed in triplicate, and duplicate controls (no yeast) were maintained. Samples (0.5 ml aliquots) were taken aseptically over time (every 24 h) and stored at −80 °C prior to composition analysis.

2.6. Analyses

Wet-basis moisture content was determined for diced roots using an oven drying method (105 °C, 24 h). Alcohol insoluble solids were measured using a modified method to estimate the initial and residual starch composition of ISPs (Ridley et al., 2005; Duvernay, 2008). Final results report the change in starch content as a fraction of the ISP dry matter, assuming the enzymes are not degrading protein and fiber (difference between initial and final AIS values). Protein and fibrous fractions of the ISP dry matter were not measured.

Total monomeric anthocyanin (TMA) content was determined using a spectrophotometric pH-differential method (Giusti and Wrolstad, 2003). The most representative anthocyanin for this investigation's TMA measurements was cyanidin-3-glucoside with a molar absorptivity (ε) of 26,900, therefore results were reported as cyanidin-3-glucoside equivalents (cyd-3-glu-E) per 100 g of fresh ISP weight (Jurd and Asen, 1966; Delgado-Vargas and Paredes-Lopez, 2003).

Total phenolic concentration was quantified using a modified spectrophotometric Folin-Ciocalteu (FC) method where chlorogenic acid was used as the standard, therefore results were reported as chlorogenic acid equivalents (CAE) per 1 g of fresh ISP weight (Singleton et al., 1999).

Sugar concentrations (maltotriose, maltose, glucose and fructose) produced after hydrolysis and consumed during fermentation, as well as ethanol produced during fermentation were measured by high performance liquid chromatography using a Biorad Aminex HPX-87H Column (Shimadzu AL-20, 65 °C, RI detector, 5 mM H_2SO_4 elution buffer, 0.6 ml/min flow rate). HPLC samples were diluted, centrifuged (14908 × *g*, 5 min) and filtered through 0.45 μm Milipore filters before analysis.

3. Results

3.1. Extraction of anthocyanins and subsequent hydrolysis of ISP residues

The analysis of variance (ANOVA) for the main and interaction effects of solvent type, extraction temperature and solid loading on total monomeric anthocyanin and phenolic concentration for purple-fleshed ISPs after extraction are shown in Table 2.

The main and interaction effects for TMA concentration were statistically significant ($P < 0.05$). TMA concentration reported the color quality of the anthocyanins present. TMA concentration over extraction temperature for all solvents at both solid loadings is shown in Fig. 1.

The highest TMA concentration of 186.1 mg cyd-3-glu/100 g fresh weight (fw) was obtained using 70% acidified methanol at 80 °C with a 3.3% (w/v) solid loading, but no statistical difference

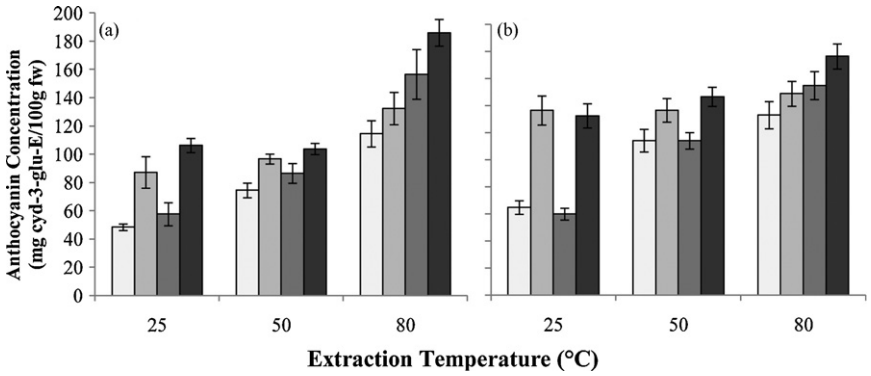

Fig. 1. TMA concentration over extraction incubation temperature for (□) 70% ethanol, (▒) 70% acidified ethanol, (▓) 70% methanol and (■) 70% acidified methanol at (a) 3.3% (w/v) and (b) 17% (w/v) solid loadings.

between solid loading was observed under the same conditions ($P > 0.05$). On average, each solvent extracted higher TMA concentrations at the higher extraction temperature of 80 °C within each solid loading than at the lower extraction temperatures of 25 or 50 °C. Solid loading was not significant for either methanol solvent at 80 °C ($P > 0.05$), but the solid loading of 17% (w/v) had greater TMA concentrations than 3.3% (w/v) solid loading for both ethanol and acidified ethanol at 80 °C ($P < 0.05$). At the lower extraction temperatures of 25 and 50 °C, acidified solvents produced statistically higher TMA concentrations than non-acidified extraction combinations within each solid loading and temperature ($P < 0.05$). Overall, acidified solvents resulted in 10–45% and 16–46% more TMA than the non-acidified solvents of ethanol and methanol, respectively. Within the acidified solvents, acidified methanol produced greater TMA concentrations on average than acidified ethanol.

The main and interaction effects for phenolic concentration were statistically significant, except for the full interaction as seen in Table 2 ($P < 0.05$). Phenolic concentration represented the overall non-flavonoid and flavonoid components (including anthocyanins) present. Phenolic concentration over extraction temperature for each solvent across solid loading is shown in Fig. 2. On average, each solvent extracted higher phenolic concentrations at 80 °C across solid loading than at 25 and 50 °C ($P < 0.05$). At 50 °C there was no statistical difference in the type of solvent used; however, for 25 °C both of the acidified solvents had statistically higher phenolic concentrations than the non-acidified solvents ($P < 0.05$). The interaction of solvent type and solid loading across temperature indicated that the higher solid loading of 17% (w/v) had statistically higher phenolic concentration than the lower solid loading of 3.3% (w/v) for all solvents ($P < 0.05$). Both methanol solvents showed statistically higher phenolic concentrations than the ethanol solvents at the lower solid loading of 3.3% (w/v) ($P < 0.05$). Overall for the 17% (w/v) solid loading, both acidified ethanol and acidified methanol showed statistically higher phenolic concentrations at 5.01 and 4.90 mg CAE/g fresh ISP, respectively, than their respective non-acidified solvents at 4.70 and 4.58 mg CAE/g fresh ISP ($P < 0.05$).

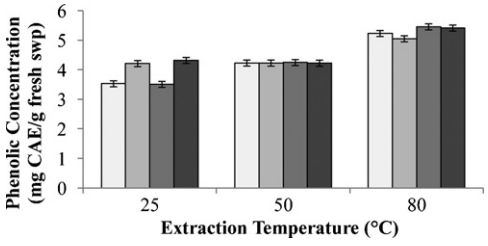

Fig. 2. Phenolic concentration over extraction temperature for (□) 70% ethanol, (▒) 70% acidified ethanol, (▓) 70% methanol and (■) 70% acidified methanol, across solid loading.

The analysis of variance (ANOVA) table for the main and interaction effects of solvent, extraction temperature, and solid loading on change in alcohol insoluble starch (AIS) and glucose concentration for purple-fleshed ISPs after extraction and hydrolysis is shown in Table 3. Change in AIS was used to represent the change in starch content as a percent of dry matter and was examined in this study to determine the amount of starch converted during hydrolysis. In this case, the main effects of solvent and extraction temperature, the interaction between solvent and temperature and solvent and solid loading, as well as the full interaction of all three factors were statistically significant ($P < 0.05$). Change in starch content as a percent of dry matter over extraction temperature for all solvents at each solid loading is shown in Fig. 3.

Initial starch content for purple-fleshed ISPs was on average 89.7% of the dry matter. Change in starch content ranged from 67 to 78.3% of the dry matter, leaving a residual starch content of at least 11.4% of the dry matter. The highest change was observed in the hydrolysis of treatments extracted with acidified ethanol using a 3.3% (w/v) solid loading at 80 °C. Acidified ethanol treatments at 80 °C showed no statistical difference between solid loadings for

Table 2
ANOVA of main and interaction effects of solvent type (Solvent), extraction temperature (Temp) and solid loading (Solid Loading) on total monomeric anthocyanin (TMA) and phenolic (phenolics) concentration for purple-fleshed ISPs after extraction.

Source	DF	TMA			Phenolics		
		MS	*F*	*P*	MS	*F*	*P*
Solvent	3	8660.3	177.23	<.0001	0.353	5.69	0.0020
Temp	2	25083.2	513.33	<.0001	12.73	205.12	<.0001
Solid Loading	1	9046.9	185.15	<.0001	7.586	122.27	<.0001
Solvent × Temp	6	1463.8	29.96	<.0001	0.492	7.93	<.0001
Solvent × Solid Loading	3	521.0	10.66	<.0001	0.410	6.60	0.0008
Temp × Solid Loading	2	1507.4	30.85	<.0001	0.956	15.41	<.0001
Solvent × Temp × Solid Loading	6	210.7	4.31	0.0015	0.041	0.66	0.6851

DF is degrees of freedom; MS is mean square; *F* is *F*-value; *P* is *P*-value.

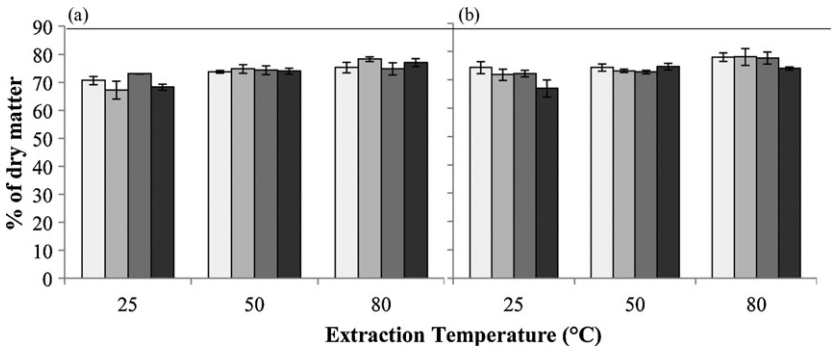

Fig. 3. Change in starch content as a percent of dry matter after hydrolysis over extraction incubation temperature for (□) 70% ethanol, (▒) 70% acidified ethanol, (▒) 70% methanol and (■) 70% acidified methanol at (a) 3.3% (w/v) and (b) 17% (w/v) solid loading treatments. Initial starch content for purple-fleshed ISPs (—).

change in starch content ($P > 0.05$). However solid loading was statistically significant for all other solvents at 80 °C where ethanol and methanol treatments resulted in greater changes in starch at 17% (w/v) solid loading and where acidified methanol performed better at 3.3% (w/v) ($P < 0.05$). Extraction did not limit the starch change relative to the controls that went through hydrolysis only (no extraction).

In hydrolysis, simple sugars such as maltotriose, maltose, glucose and fructose can be generated from the enzymatic conversion of starch. For all treatments in this investigation, the primary sugar generated from hydrolysis was glucose. No maltotriose or fructose was present and only trace amounts of maltose were observed. This could be due to the effect of the initial presence of solvent and extended incubation in a high temperature environment on the ISP structure, as the incubation temperatures were close to optimal for activity of the hydrolysis enzymes used and naturally present in the root. Previous studies that showed maltotriose and maltose still present after hydrolysis (data not shown) only subjected the ISP to contact with enzymes and high temperature for the duration of the hydrolysis process. Thus, it seems that the initial extraction process may have enhanced enzyme activity toward the conversion of these polysaccharides to glucose.

The main effects of solvent and extraction temperature as well as the interaction between the two were statistically significant for glucose concentration after hydrolysis ($P < 0.05$). Glucose concentrations after hydrolysis for all solvents over extraction temperature and across solid loading are shown in Fig. 4. Glucose concentrations resulting from hydrolysis in treatments that went through extraction ranged from 268 to 395 mg/g dry ISP. Controls that were treated with hydrolysis conditions only (no extraction) produced statistically higher amounts of glucose on average (488.7 mg/g dry ISP) than any of the treatments that went through extraction and hydrolysis ($P < 0.05$). The highest glucose concentration from treatments was observed after using acidified ethanol at 50 °C (379.6 mg/g dry ISP); however, this was not statistically different than glucose resulting from ethanol (25, 50 °C) and acidified methanol (25 °C) extraction conditions ($P > 0.05$). On average, treatments with methanol had lower glucose concentrations compared to the ethanol based solvents.

3.2. Starch conversion and ethanol production from ISP extraction residues

Since the majority of sugars produced from residual solids that underwent anthocyanin extraction were in the form of the simple sugar glucose, the fermentability of the produced glucose was examined by analyzing sugar consumption as well as ethanol production during fermentation. Glucose concentrations after hydrolysis for the two enzyme loadings over extraction conditions is shown in Fig. 5. For the 80 °C extraction conditions, the higher enzyme loading of 5.0 AGU/g dry ISP produced greater amounts of glucose on average than the lower enzyme loading within each extraction condition. Out of the three extraction conditions chosen, acidified ethanol at 50 °C resulted in the highest glucose concentration of 432 mg/g dry ISP on average across enzyme loading. This extraction condition also produced statistically higher concentrations of glucose than the controls that went through hydrolysis only, which only produced 394.9 mg/g dry ISP

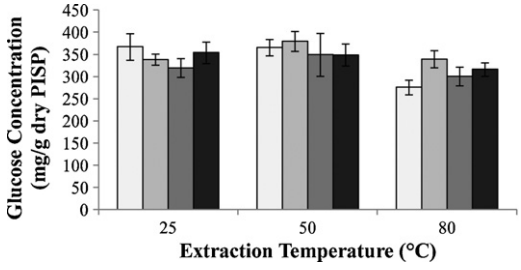

Fig. 4. Glucose concentration after hydrolysis over extraction temperature for (□) 70% ethanol, (▒) 70% acidified ethanol, (▒) 70% methanol and (■) 70% acidified methanol, across solid loading.

Table 3
ANOVA of main and interaction effects of solvent (Solvent), extraction temperature (Temp) and solid loading (Solid Loading) on change in alcohol insoluble starch (ΔAIS) and glucose concentration (Glucose) for purple-fleshed ISPs after extraction and hydrolysis.

Source	DF	ΔAIS			Glucose		
		MS	*F*	*P*	MS	*F*	*P*
Solvent	3	12.94	4.48	0.0075	2626.5	4.21	0.0101
Temp	2	223.7	77.38	<.0001	17636.1	28.25	<.0001
Solid Loading	1	5.888	2.04	0.1600	139.7	0.22	0.6383
Solvent × Temp	6	15.29	5.29	0.0003	2736.2	4.38	0.0013
Solvent × Solid Loading	3	10.00	3.46	0.0234	960.7	1.54	0.2166
Temp × Solid Loading	2	6.187	2.14	0.1287	197.6	0.32	0.7302
Solvent × Temp × Solid Loading	6	8.964	3.10	0.0120	431.7	0.69	0.6576

DF is degrees of freedom; MS is mean square; *F* is *F*-value; *P* is *P*-value.

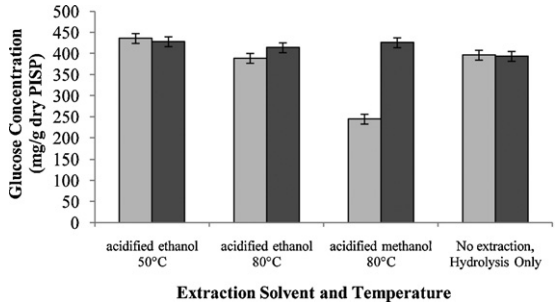

Fig. 5. Glucose concentration over extraction conditions for (■) 2.5 and (■) 5.0 AGU/g dry ISP after hydrolysis.

on average ($P < 0.05$). All other extraction conditions showed no significant difference ($P > 0.05$) in glucose concentration than the controls that went through hydrolysis only, except for acidified methanol at 80 °C with 2.5 AGU/g dry ISP enzyme loading which was significantly smaller at 244.5 mg/g dry ISP ($P < 0.05$).

Ethanol from produced sugars was examined to determine the fermentation potential of purple-fleshed ISP sugars derived after extraction and hydrolysis processing. Glucose concentration and ethanol production over fermentation incubation time for 5.0 AGU/g dry ISP enzyme loading, and all extraction conditions are shown in Fig. 6. The enzyme loading of 5.0 AGU/g dry ISP as shown is representative of the glucose consumption and ethanol production trends for both enzyme loading rates studied.

Controls that did not go through an initial extraction before hydrolysis exhausted all glucose present after 60 h of fermentation and produced 42 g/l of ethanol. Between 24 and 48 h glucose consumption was minimal with 10.07%, 9.89% and 15.4% consumed by treatments extracted with acidified ethanol at 50 °C, acidified ethanol at 80 °C, and acidified methanol at 80 °C, respectively. This is compared to 63.84% reduction in glucose concentration observed in controls that did not go through an initial extraction. The rate of glucose consumption between 48 and 72 h ranged between 0.31 and 0.47 g/l/h, but still was not as high as 1.86 g/l/h for controls.

The rate of ethanol production between 24 and 48 h was 0.07 and 0.14 g/l/h for acidified ethanol at 50 °C and 80 °C, respectively, compared to 0.92 g/l/h for controls. Acidified ethanol at 50 °C using 5.0 AGU/g dry ISP had the highest ethanol produced of all the pre-extraction treatments at 38.5 g/l (120 h). Acidified methanol at 80 °C did not produce any fermentation products until after 48 h of fermentation, resulting in a maximum ethanol production of 28.7 g/l (Fig. 6).

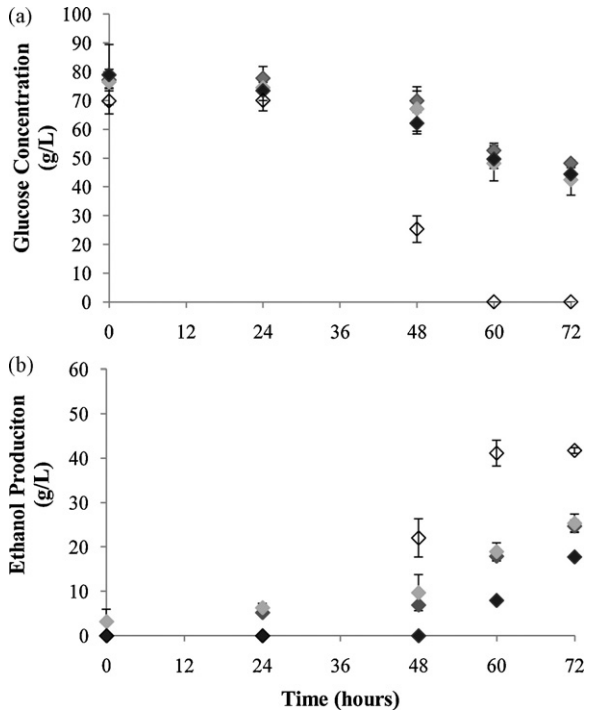

Fig. 6. (a) Glucose concentration and (b) ethanol production within the 5.0 AGU/g dry ISP enzyme loading over fermentation incubation time for extraction conditions of (◊) acidified ethanol at 50 °C, (◆) acidified ethanol at 80 °C, (♦) acidified methanol at 80 °C, (◊) no extraction fermentation control.

4. Discussion

Extracted anthocyanins from purple-fleshed sweetpotatoes have been reported in literature ranging from 15 mg/100 g fw to 182 mg/100 g fw (Brown et al., 2005; Cevallos-Casals and Cisneros-Zevallos, 2003). Initial extraction of purple-fleshed ISPs in this study produced anthocyanin concentrations comparable to studies in literature, providing maximum results of 186.1 mg cyanidin-3-glu/100 g fw. Anthocyanin recovery in this work was higher than those results found in Teow et al. (2007) and Brown et al. (2005). Teow et al. (2007) showed purple-fleshed sweetpotato varieties ranging in 24.6–43.0 mg/100 g fw where sweetpotatoes were freeze dried into powder and extracted first with hexane for lipophilic antioxidants and then subsequently extracted with acidified methanol at room temperature for anthocyanins. Brown et al. (2005) found lower anthocyanin concentrations ranging from 15 to 38 mg/100 g fw using purple-fleshed potatoes that were frozen immediately in liquid nitrogen, ground to a powder, and then extracted with a 70% acetone water mixture incubating in a hot water bath. Other investigations showed TMA results in the same range as this study. Steed and Truong (2008) found ranges of 84–174 mg/100 g fw using an accelerated solvent extractor with another purple-fleshed ISP clone. In addition, Cevallos-Casals and Cisneros-Zevallos (2003) reported an anthocyanin content of a red-fleshed sweetpotato cultivar of 182 mg anthocyanin/100 g fw after sample homogenization with an ethanolic solvent (0.225N HCl in 95% ethanol) in an extended extraction incubation of 24 h at 4 °C.

Total monomeric anthocyanin results showed greater yields at the higher extraction temperature of 80 °C. Fan et al. (2008) also observed that anthocyanin yield can be increased with an increase in extraction temperature. It was also evident in both Fan et al. (2008) and this study that extraction temperature and solid loading separately affect anthocyanin yield, while extraction time was insignificant. However, Fan et al. (2008) found that a lower solid loading of 1:32 (solid–liquid ratio) performed better than all other solid loadings investigated that ranged from 1:15 to 1:35. The data presented in this work showed no significant difference in anthocyanin yield between the lower (1:30) and higher (1:6) solid loadings, suggesting that a high amount of solvent is not needed for a meaningful recovery of anthocyanins.

Acidified solvents (pH ~ 3.5) also performed better in anthocyanin recovery than non-acidified at temperature of 80 °C. This is related to the functional properties of anthocyanins where they have greater stability under acidic conditions (Tair et al., 1999; Kong et al., 2003; Delgado-Vargas and Paredes-Lopez, 2003). Anthocyanins are stabile at a pH between 1 and 3, but at pH >4 the structure is not stable and could undergo transformation. Fan et al. (2008) observed this occurrence when the anthocyanins recovered in purple sweetpotato powder were more stable under the acid conditions between pH 2.0 and 4.0 than the slightly acid conditions between pH 5.0 and 6.0. Thus some research groups incorporated the use of acidic solvents that contain small amounts of hydrochloric acid or formic acid (Tair et al., 1999; Delgado-Vargas

and Paredes-Lopez, 2003; Kong et al., 2003). It was also observed in this study that acidified solvents had the same recovery benefit at lower extraction temperatures suggesting that both solvent acidification and high temperature are key factors to anthocyanin yield.

Overall, methanol solvents performed the best in this investigation in extracting anthocyanins compared to ethanol solvents. Ethanol and methanol extracts of purple-fleshed ISPs did have approximately three to four times higher values of phenolics and anthocyanins compared to water extracts obtained during the conversion process in a previous study (data not shown). Lapornik et al. (2005) explored this in their study where anthocyanin characteristics were examined. It was found that solvent effectiveness is related to system polarity. Anthocyanins are naturally polar compounds, therefore their recovery would be more effective in solvents of similar polarity. Methanol and ethanol, relative to water, have similar characteristics to anthocyanins making them better suited for extraction. There was a difference in the concentrations observed using methanol and ethanol. The higher concentrations resulting from the use of methanol may be due to its smaller size offering more opportunity of reaching areas ethanol cannot (Pankaj and Sharma, 1991). However, the characteristics of ethanol as a solvent are more desirable in the food industry than methanol, suggesting ethanol may show more promise as an extraction solvent for food-based applications.

Between the two extraction and hydrolysis investigations, an increase in glucose was observed in treatments that went through both extraction and hydrolysis and may be attributed to the additional washing cycle that was incorporated into the second study. Washing is a significant step after extraction in order to remove the solvent present prior to enzymatic hydrolysis. This is necessary because in previous studies (data not shown) residual solvent during hydrolysis showed a negative effect on the enzyme's ability to break down the starch to sugars. Additional washing may improve hydrolysis conditions; however, it should also be considered that free sugars in the liquid may be lost during washing. Excess solvent also had a negative effect on yeast fermentation, thus establishing a complete process for extraction with subsequent hydrolysis and fermentation will require attention to efficient removal of residual solvent.

5. Conclusions

Anthocyanin extracts and fermentable sugars can be obtained as co-products through an integrated process. After testing various liquids to aid in extraction, it was clear that the extraction of total monomeric anthocyanin and phenolics was greater with the use of solvents than with water. Methanol solvents showed a statistically higher performance in anthocyanin and phenolic recovery than ethanol solvents. However ethanol may be a suitable alternative considering the ethanol product may be obtained from subsequent hydrolysis and fermentation, making a recyclable process. Although methanol solvents had higher anthocyanin and phenolic recovery, they showed lower fermentable sugar production than ethanol solvents. Overall, it is possible to extract anthocyanin and phenolic compounds from purple-fleshed ISPs while maintaining available starch for hydrolysis, making it a promising substrate for development of industrial colorants and dyes.

Acknowledgements

The authors would like to thank Novozymes North America, Inc. (Franklinton, NC) and Fermentis, Lesaffre Yeast Corporation for their donations, Dr. Craig Yencho and Mr. Ken Pecota (Sweetpotato Breeding Program, NCSU) for supply of ISPs, and Dr. Mike Boyette for the use of post-harvest processing equipment during experimentation. The authors would also like to extend a special thanks to William Duvernay, Roger Thompson, Laurie Steed and Amy Byrd for their assistance in experimentation and lab work.

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