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ARTICLE

Release of Bound Procyanidins from Cranberry Pomace by Alkaline Hydrolysis

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Procyanidins in plant products are present as extractable or unextractable/bound forms. We optimized alkaline hydrolysis conditions to liberate procyanidins and depolymerize polymers from dried cranberry pomace. Alkaline extracts were neutralized (pH 6−7) and then procyanidins were extracted with ethyl acetate and analyzed by normal phase high performance liquid chromatography. Alkaline hydrolysis resulted in an increase in low molecular weight procyanidins, and the increase was greater at higher temperature, short time combinations. The most procyanidins (DP1−DP3) were extracted at 60 °C for 15 min with each concentration of NaOH. When compared to conventional extraction using homogenization with acetone/water/acetic acid (70:29.5:0.5 v/v/v), treatment with NaOH increased procyanidin oligomer extraction by 3.8−14.9-fold, with the greatest increase being DP1 (14.9×) and A-type DP2 (8.4×) procyanidins. Alkaline treatment of the residue remaining after conventional extraction resulted in further procyanidin extraction, indicating that procyanidins are not fully extracted by conventional extraction methods.

KEYWORDS: Alkaline; cranberry; hydrolysis; pomace; procyanidins; unextractable

INTRODUCTION

Cranberries (*Vaccinium macrocarpon*) are growing in popularity due to the increasing information regarding their health benefits. Cranberry juice has long been recognized for its ability to prevent urinary tract infections; however, there are several other health benefits associated with cranberries, which include antioxidant, antitumor, antiulcer, anti-inflammatory, and antiatherosclerotic activities (*1*−*4*). Cranberry pomace is composed primarily of seeds, skins, and stems, which are leftover from the juicing and canning processes of the cranberry processing industry (*5*). The seeds and skins of berries are a rich source of polyphenolic compounds, which have shown to be responsible for the numerous health benefits associated with the berries.

Procyanidins are a class of polyphenolic compounds that impart astringency and bitterness to many plant products. In plants, they are believed to serve as a defense mechanism against potential predators because their bitterness and astringency is undesirable to animals, insects, and microbes (*6*). Procyanidins are formed via the condensation of the flavan-3-ols catechin and epicatechin and consist of two to several monomeric units (*6*). Structurally, the monomeric units may be linked in one of three ways. The "B"-type linkage is the most common and consists of 4$\beta \rightarrow$ 8 linkage between units. Units connected by both a 2$\beta \rightarrow$ O-7 and a 4$\beta \rightarrow$ 8 linkage are more rigid than "B"-type linkages and are denoted as "A"-type. The final type of linkage is the "C"-type linkage, which consists of a C-4 → C-6 linkage (*6*). Recently, the ability of cranberries to prevent urinary tract infections has been attributed to the presence of procyanidins containing "A"-type linkages (*7*). The bioavailability of procyanidins is dependent upon the size of the molecule with monomers and dimers being absorbed and present in blood at relatively low levels, but those larger than trimers are not absorbed (*8*, *9*). Whether absorption is required for procyanidins to impart their health benefits is still unknown.

Polyphenolic compounds, including procyanidins, are commonly perceived to be found mainly in the vacuoles of plants where they are separated from other cellular components. However, many may also be associated with cellular components, such as the cell wall, especially after cell injury when vacuoles may rupture. This results in the release of phenolic compounds which may then associate with cell wall polysaccharides through hydrogen bonding and hydrophobic interactions (*10*). Procyanidins in particular have a strong affinity for cell wall material (*11*), with higher molecular weight compounds having a greater affinity for binding than smaller compounds. The idea of "unextractable" procyanidins has been of great interest recently because it is believed that the procyanidin contents in plant materials has been underestimated due to the presence of procyanidins bound so tightly to cell wall material that they are not released by normal extraction methods (*12*−*16*).

Alkaline treatments are commonly used to extract bound phenolic acids and other phenolic compounds from grains such as rice, wheat, and corn. It is known that phenolic compounds, namely, ferulic acid, are insoluble and bound to cell wall materials. Treatment with different concentrations of sodium hydroxide for varying lengths of time has proven to be effective in releasing these bound phenolic compounds (*17*, *18*). There is

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limited information, however, on the effectiveness of alkaline treatment to release bound phenolic compounds in fruits possibly because many phenolic compounds in fruits, including anthocyanins, are known to be unstable under alkaline conditions. Furthermore, there is even less research regarding the possible release of procyanidins from fruit and vegetables by alkaline treatment. Researchers have shown that strong alkaline conditions can result in cleavage of the C−C interflavan bond connecting the monomeric units of procyandins; however, prolonged treatment can cause further degradation by opening of the A-ring of the flavan-3-ol. Research regarding the effect of alkaline conditions on procyanidins has been limited to purified compounds (*19*, *20*). Additionally, the effects of alkaline conditions on A-type linkages common in cranberries have yet to be detailed. We have recently reported that cranberry pomace contains significant levels of procyanidins with primarily A-type linkages. In this paper, we report on the efficacy of sodium hydroxide treatment in releasing bound procyanidins from cranberry pomace in the form of low molecular weight monomers and oligomers, thus providing a valuable source of procyanidins with potential biological activity.

MATERIALS AND METHODS

Chemicals and Standards. HPLC-grade acetone, methanol, acetonitrile, ethyl acetate, acetic acid, and formic acid were obtained from EMD Biosciences (Madison, WI). Sephadex LH-20 was purchased from Sigma Chemical Co. (St. Louis, MO). Sodium hydroxide was purchased from Fisher Scientific (Pittsburgh, PA). Procyanidin standards derived from cocoa (DP1−DP9) were obtained from Masterfoods Inc., (Hackettstown, NJ).

Sample. Dried cranberry pomace was obtained from Decas Cranberry Company (Carver, MA) and stored at −20 °C until use. The pomace was ground to pass through a 1000 μm sieve screen using an Udy Cyclone Sample Mill (Fort Collins, CO) and stored at −70 °C.

Alkaline Treatment of Cranberry Pomace. Ground cranberry pomace (0.5 g) was weighed and placed into glass, screw-top tubes. Five mL of 2 N, 4 N, or 6 N NaOH was added to the tubes, and the tubes were then flushed with nitrogen for 30 s, capped, and vortexed. Tubes were then placed in a shaking water bath (200 rpm) set at 25, 40, or 60 °C for 5 min to 24 h depending on the temperature. After tubes were removed from the water bath, they were placed in an ice bath, and their pH was adjusted to 6−7 using 4 N HCl.

Alkaline Treatment of Residue Following Conventional Solvent Extraction. Dried cranberry pomace (0.5 g) was extracted using the homogenization method described below. After three extractions, the residue was collected, and the excess acetone was removed using a SpeedVac concentrator (ThermoSavant, Holbrook, NY). The residue was then alkaline treated using 5 mL of 2 N NaOH for 15 min at 60 °C. The pH was adjusted to 6−7 using 4 N HCl, and procyanidins were extracted as described below.

Extraction of Procyanidins. Neutralized samples from the alkaline treatment of pomace were transferred to 250 mL plastic bottles. Lipids were extracted by shaking with hexane (40 mL) and centrifuging for 10 min at 10864*g*; the lipid fraction was discarded. Procyanidin monomers, dimers, and trimers were extracted with ethyl acetate (40 mL) and centrifuged for 10 min at 10864*g*. Ethyl acetate extraction was repeated, and the extracts were pooled.

A separate extraction method was used to extract procyanidin monomers through polymers. This was performed using a T18 Basic Ultra-Turrax homogenizer (IKA WORKS, Wilmington, NC, USA). Neutralized samples were mixed with 20 mL of acetone/water/acetic acid (AWA, 70:29.5:0.5 v/v/v), homogenized for 1 min, and filtered through Miracloth. The extraction was repeated two more times, the extracts were pooled, and volume was adjusted to 100 mL with extraction solvent.

Although extraction with ethyl acetate only allowed quantification of monomers, dimers, and trimers, it provided a quick means to screen extraction conditions because it required no further cleanup step. Once an ideal condition was determined, higher oligomers were extracted using homogenization with AWA which extracts procyanidin monomers through polymers (DP ≥ 10).

Table 1. Treatment Conditions for Alkaline Hydrolysis

temperature (°C)	time	concentration (N)
25	1 h	2, 4, 6
	3 h	2, 4, 6
	6 h	2, 4, 6
	12 h	2, 4, 6
	24 h	2, 4, 6
40	30 min	2, 4, 6
	1 h	2, 4, 6
	1.5 h	2, 4, 6
	2 h	2, 4, 6
60	5 min	2, 4, 6
	10 min	2, 4, 6
	15 min	2, 4, 6
	30 min	2, 4, 6

Sephadex LH-20 Isolation of Procyanidins. Procyanidins extracted by homogenization with AWA were isolated from sugars and other phenolic compounds by solid phase extraction using Sephadex LH-20 according to the method described by Gu et al. (*21*).

Purification of Polymeric Procyanidins from Cranberry Pomace. Twenty grams of cranberry pomace were extracted with 500 mL of acetone/water/acetic acid (70:29.5:0.5 v/v/v) by homogenization. The extract was divided into 25 mL aliquots, and acetone was evaporated from them using a vacuum concentrator. The extracts were then manually loaded onto a column containing 3 g of hydrated Sephadex LH-20. Sugars and other phenolics were eluted from the column with 30% aqueous methanol, and monomers and procyanidin oligomers were eluted with 100% methanol. Polymeric procyanidins were then eluted with 70% aqueous acetone, and this fraction was collected. Acetone was removed from the polymer fraction using a vacuum concentrator, and the remaining aqueous portion was freeze-dried to obtain a light pink powder.

Alkaline Treatment of Purified Polymeric Procyanidins. Polymeric procyanidins (10 mg) were alkaline treated for 15 min at 60 °C using 1 mL of 2 N NaOH. The pH was adjusted using 4 N HCl, and the total volume was adjusted to 5 mL with acetone/water/acetic acid solvent. Procyanidins were then directly analyzed by HPLC.

HPLC Analysis of Procyanidins. Ethyl acetate extracts and extracts resulting from LH-20 isolation were evaporated to dryness using a SpeedVac concentrator, resuspended in 2 mL of AWA and filtered through 0.45 μm filters for HPLC analysis. Procyanidins were separated using the method of Hammerstone et al. (*22*) with modifications as described previously (*23*). Procyanidins were quantified using a mixture of standards (DP1−DP10) isolated from cocoa (*24*). A-type procyanidins were quantified as B-type equivalents. Identification of the procyanidins in cranberry pomace by LC-MS and MALDI-TOF-MS was previously reported (*23*).

Experimental Design and Analysis. A split-plot randomized block design was used for treatment application with water bath temperature as the whole plot and sodium hydroxide concentration as the split plot. Time was nested within temperature, which is denoted as time [temperature]. A single treatment consisted of one temperature, one time, and one sodium hydroxide concentration. Three water bath temperatures (25, 40, 60 °C) and three sodium hydroxide concentrations (2, 4, and 6 N) were evaluated. Time of treatment varied depending on temperature. There were a total of 39 treatments (**Table 1**), and levels of each factor were randomized within each plot.

Analysis of variance and mean separations were determined by the PROC MIXED procedure using SAS (SAS 9.1, SAS Institute, Cary, NC). Differences between means were determined using the protected LSD ($\alpha = 0.05$).

RESULTS

Alkaline Treatment of Cranberry Pomace. Cranberry pomace was treated with varying concentrations of sodium hydroxide at different temperatures for different amounts of time. Monomeric (DP1), dimeric (DP2), and trimeric (DP3) procyanidins were extracted from the alkaline treated pomace. The overall analysis of variance is presented in **Table 2**. When averaged over all

Table 2. Analysis of Variance for Procyanidin Extraction Using Sodium Hydroxide

source	df	*F*-value	*P*
	monomer		
temperature	2	89.38	<0.0001
time [temperature]	10	3.59	0.0050
normality	2	1.13	0.3292
normality × temperature	4	0.81	0.5270
normality × time [temperature]	20	1.88	0.0351
	dimer		
temperature	2	67.59	<0.0001
time [temperature]	10	5.70	0.0002
normality	2	2.75	0.0731
normality × temperature	4	0.93	0.4564
normality × time [temperature]	20	2.11	0.0160
	trimer		
temperature	2	20.37	<0.0001
time [temperature]	10	3.74	0.0039
normality	2	0.69	0.5054
normality × temperature	4	0.42	0.7910
normality × time [temperature]	20	1.78	0.0489

Table 3. Procyanidin (DP1-DP3) Concentration (mg/100 g DW) of Cranberry Pomace Treated with Sodium Hydroxide at Different Temperatures

temperature (°C)	DP1	DP2	DP3
25	44.6 ± 1.9b[a,b]	248.9 ± 12.8c	85.6 ± 4.5c
40	84.8 ± 3.1a	420.3 ± 11.8b	114.7 ± 4.3b
60	85.5 ± 2.6a	519.3 ± 22.4a	144.5 ± 9.2a

[a] Values represent means ± standard error. [b] Values within each column followed by the same letters are not significantly different ($p > 0.05$).

normalities and times, the effect of temperature was significant for DP1−DP3 procyanidins, and these results are presented in **Table 3**. Monomers were extracted better at 40 and 60 °C than at 25 °C. Dimers and trimers were extracted best at 60 °C, followed by 40 and 25 °C. Generally, higher temperatures resulted in increased extraction of procyanidins.

The highest order reaction which showed significance for DP1−DP3 procyanidins was normality × time [temperature]. The significance of this interaction indicates that release of procyanidins at certain time/temperature combinations is different depending on the normality of sodium hydroxide used. Comparisons were made among all time − temperature − normality combinations. The effects of temperature and normality over time for DP1 release is presented in **Figure 1**. Several conditions yielded the highest amount of monomers (DP1), and all of the conditions were at either 40 or 60 °C. The effects of temperature and normality over time for DP2 release is presented in **Figure 2**. Overall, dimer (DP2) release was greatest at 60 °C for 15 min using all three concentrations of NaOH. The effects of temperature and normality over time for DP3 release is presented in **Figure 3**. When comparing all conditions, trimer (DP3) release was greatest at 60 °C for 15 min using 2 and 4 N NaOH. Since cranberry pomace contains more dimers than other procyanidin oligomers, the ideal extraction condition was chosen based on dimer extraction. Therefore, 60 °C for 15 min was chosen as the "best" condition to release procyanidins. Since, at this temperature, there were no differences in NaOH concentrations, the lowest concentration (2 N) was chosen, and this condition was used for further experiments.

Procyanidins extracted from cranberry pomace treated with NaOH were compared to those extracted using conventional extraction by homogenization, and these results are presented in **Figure 4**. Higher amounts of procyanidins were extracted from the pomace following NaOH treatment compared to conventional extraction. HPLC chromatograms of procyanidins in cranberry pomace before and after treatment with NaOH are presented in **Figure 5**. The procyanidins in the pomace were previously identified by LC-MS and MALDI-TOF-MS (*23*). Procyanidin monomers (DP1) and oligomers (DP2−DP6) were extracted at higher levels after treatment with NaOH. The increases were most evident in DP1 (14.9-fold) and DP2 (8.4-fold) procyanidins. In total, treatment with NaOH resulted in a 9.4-fold increase in procyanidins of DP1−DP6. Homogenization with AWA also allowed for extraction of polymeric procyanidins (DP ≥ 10). There was a reduction in polymeric procyanidins in pomace treated with NaOH (518.9 mg/100 g DW) compared to conventional extraction (1188.6 mg/100 g). Including polymeric procyanidins, alkaline hydrolysis resulted in a 30% increase in total procyanidins compared to conventional extraction with 1685 mg/100 g DW and 1292 mg/100 g DW extracted, respectively. MALDI-TOF-MS was used to confirm the presence of DP2−DP6 procyanidins in the alkali treated pomace using previously described conditions (*21*). The ethyl acetate fraction was found to contain dimers (*m*/*z* 599) and trimers (*m*/*z* 887), while the AWA extract contained dimers, trimers, tetramers (*m*/*z* 1173), pentamers (*m*/*z* 1461), and hexamers (*m*/*z* 1749).

Alkaline Treatment of Residue Following Conventional Solvent Extraction. To estimate the amount of bound procyanidins in cranberry pomace, anthocyanins, flavonols, and "free" procyanidins were extracted from cranberry pomace by homogenization with AWA, and the resulting residue was collected and treated with 2 N NaOH at 60 °C for 15 m. We have previously identified and quantified the anthocyanins, flavonols, and procyanidins obtained by conventional extraction of the pomace (*23*). The amount of procyanidin oligomers further extracted from the residue after NaOH treatment is shown in **Figure 4**. Treatment of the residue with NaOH resulted in further extraction of procyanidins that were not released by conventional extraction. In total, 716.4 mg/100 g DW procyanidins with DP1−DP6 were released from the residue compared to 165.7 mg/100 g DW that were extracted by the conventional method. The released procyanidins were primarily in the form of monomers, dimers, and other lower oligomers.

Alkaline Treatment of Purified Polymeric Procyanidins. Polymeric procyanidins isolated from cranberry pomace were treated with sodium hydroxide under the optimized conditions to estimate the contribution of depolymerization in our observed increase in low molecular weight procyanidins. **Figure 6** shows the HPLC chromatograms of the purified polymer before and after alkaline treatment. It is clear that the polymer was depolymerized to primarily monomers and dimers. On a weight basis, however, approximately 5% of the polymer was converted to dimer and less than 1% was converted to monomers. The identity of the dimer was confirmed as an A-type by MALDI-TOF-MS (*m*/*z* 599).

DISCUSSION

Alkaline Treatment of Cranberry Pomace. Treatment of cranberry pomace with NaOH effectively enhanced the extraction of procyanidin monomers and oligomers. This was coupled with a significant loss in polymeric procyanidins. An increase in reaction temperature allowed for enhanced extraction of DP1−DP3 procyanidins. Additionally, the time needed for procyanidins to be released was much lower at 60 °C (15 m) than at 25 °C (> 24 h). Under harsher conditions (e.g., longer treatment times, higher temperatures), procyanidin yields were lower, indicating degradation. It appears that, in this experiment, release of procyanidins was accompanied

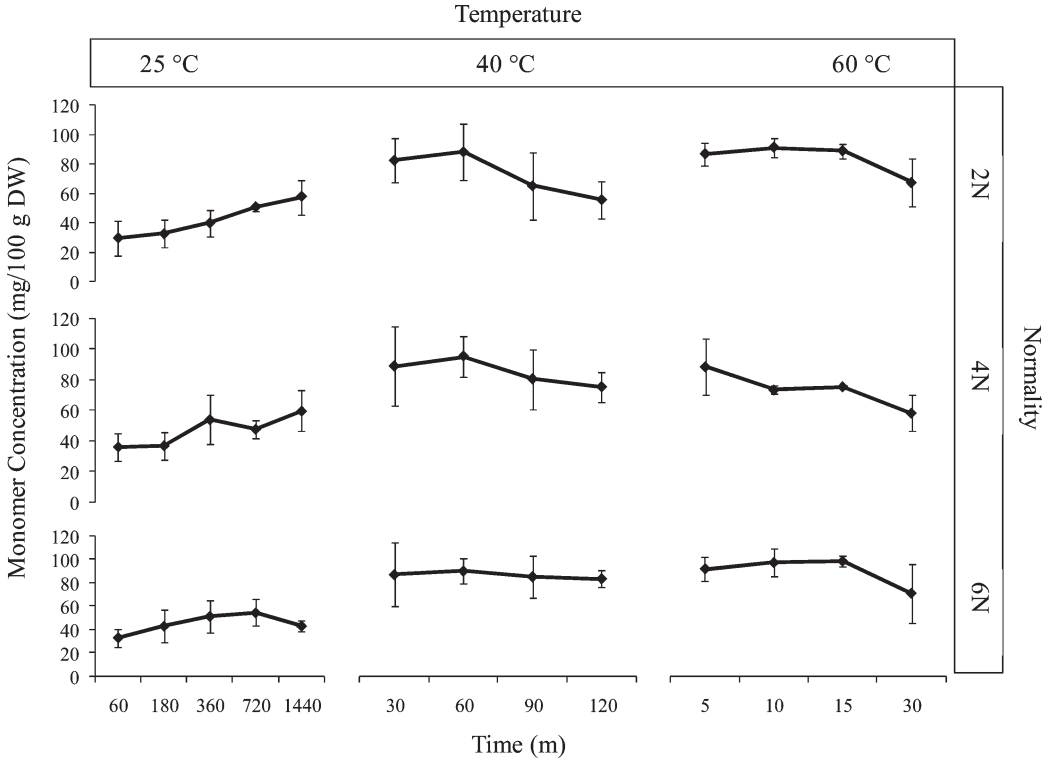

Figure 1. Changes in procyanidin monomer (DP1) composition of cranberry pomace treated with different concentrations of sodium hydroxide at different temperatures for varying amounts of time.

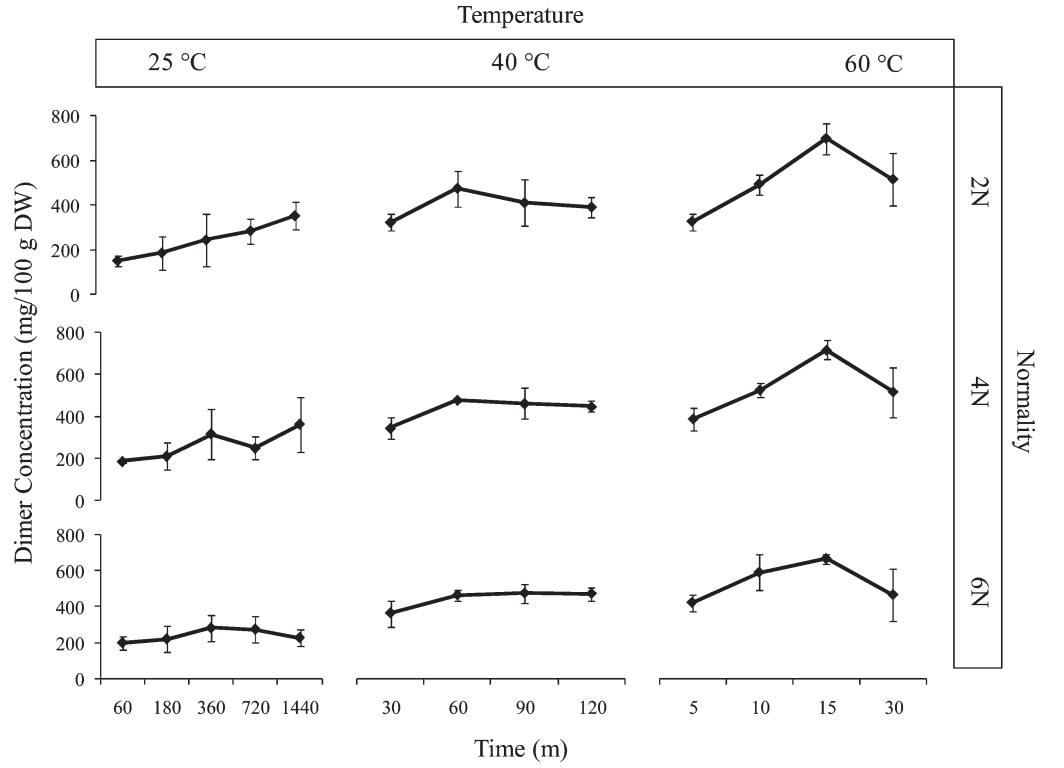

Figure 2. Changes in procyanidin dimer (DP2) composition of cranberry pomace treated with different concentrations of sodium hydroxide at different temperatures for varying amounts of time.

by procyanidin degradation; however, at the optimum condition identified in this study, we observed a significant increase in procyanidin extraction unlike any other that has previously been reported. The mechanism by which alkaline conditions resulted in such a significant increase in low molecular weight procyanidins is not fully understood, but it is likely a combination of depolymerization of polymeric procyanidins through cleavage of the C−C interflavan bond and enhanced extraction of bound procyanidins.

Depolymerization of Polymeric Procyanidins. We isolated polymeric procyanidins from cranberry pomace and treated them

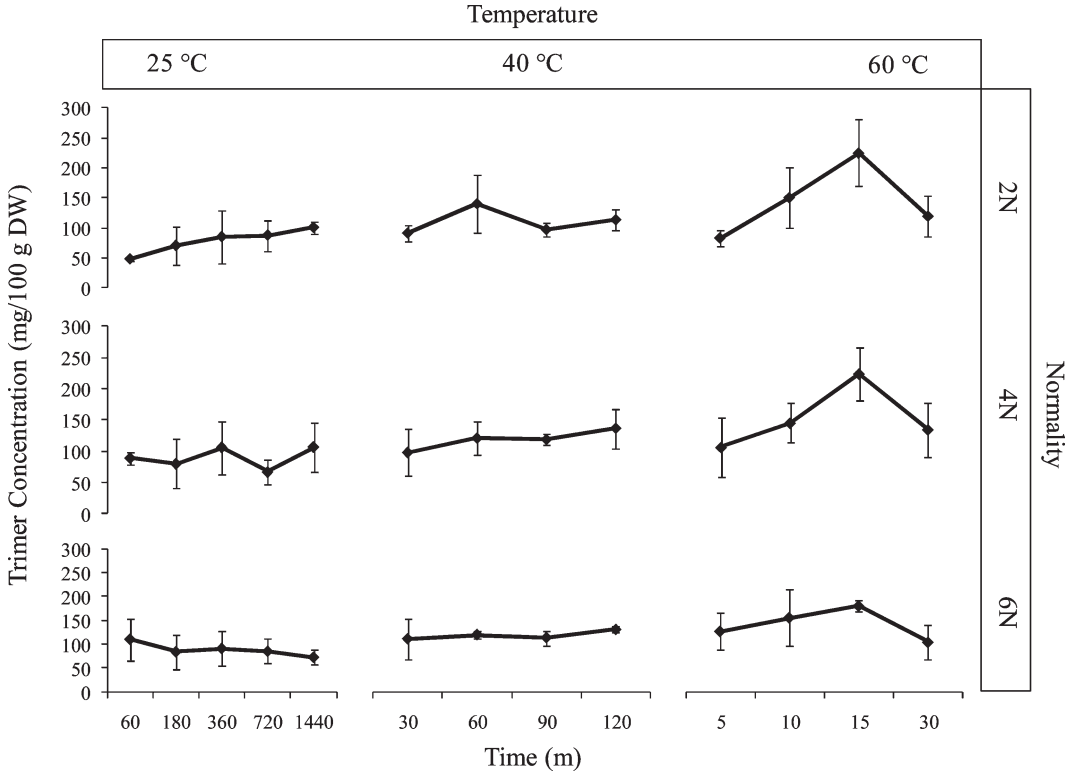

Figure 3. Changes in procyanidin trimer (DP3) composition of cranberry pomace treated with different concentrations of sodium hydroxide at different temperatures for varying amounts of time.

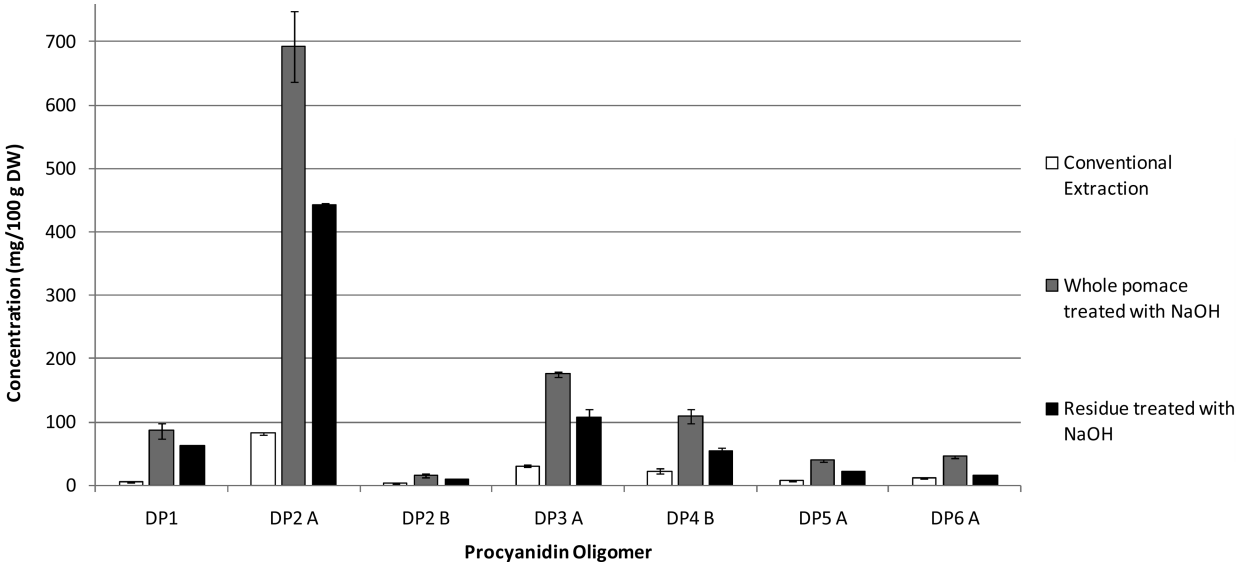

Figure 4. Procyanidin oligomer (DP1−DP6) composition of cranberry pomace before and after treatment with sodium hydroxide. Treatment conditions were 2 N NaOH at 60 °C for 15 m. Residue was collected following conventional extraction by homogenization with acetone/water/acetic acid (70:29.5:0.5). "DPn A" indicates a procyanidin containing at least one A-type linkage, and "DPn B" indicates a procyanidin containing only B-type linkages.

with sodium hydroxide under the optimized conditions. In doing so, we observed that depolymerization of the polymer to lower molecular weight procyanidins occurred in isolated compounds. However, only a small percentage of the polymer was actually converted, while the remainder was likely degraded. It has been previously demonstrated that alkaline conditions are capable of breaking the C−C interflavan bonds of procyanidins from pine bark similar to the way in which they are cleaved under acidic conditions (*19*). However, these studies have only been conducted on isolated compounds, and hydrolysis is generally performed in the presence of various nucleophiles such as bezylmercaptan or phloroglucinol, which quench the carbocation formed as each extension unit is released from the polymer, and this results in the formation of flavan-3-ols with nucleophilic adducts (*20*). When the pomace and residue were treated with alkali, it is likely that high pH in combination with elevated temperatures resulted in depolymerization of polymeric procyanidins to monomers and other oligomers. Alkaline conditions can also cause degradation of flavan-3-ols and procyanidins by opening of the A-ring leading to the formation of various side products such as catechinic acid;

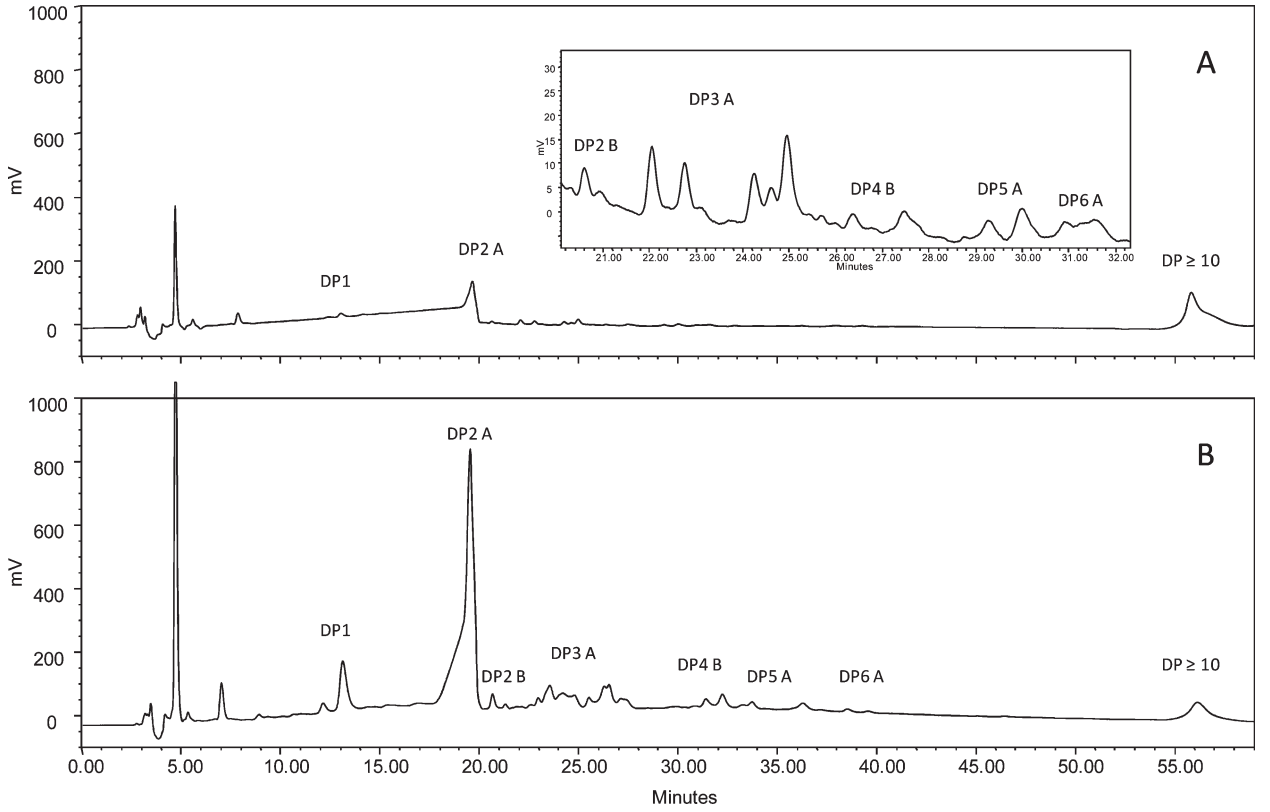

Figure 5. HPLC chromatograms of procyanidins in cranberry pomace before (A) and after (B) treatment with sodium hydroxide. Treatment conditions were 2 N NaOH at 60 °C for 15 min.

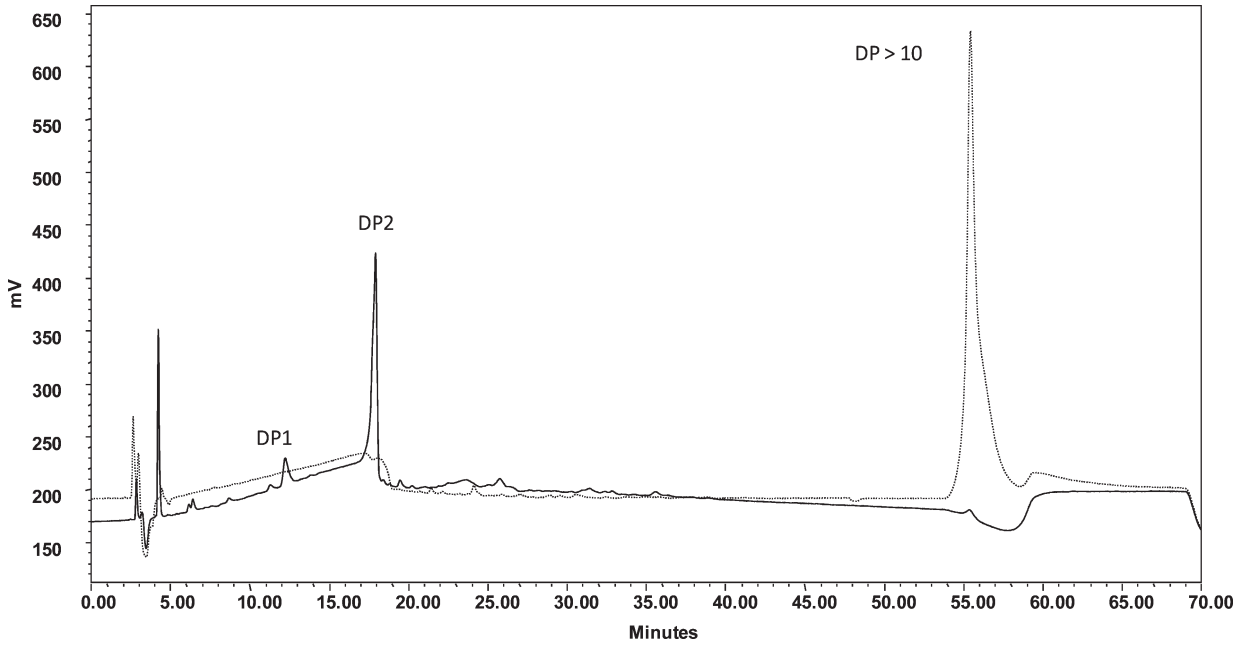

Figure 6. HPLC chromatograms of purified polymeric procyanidins from cranberry pomace before (dotted line) and after treatment with sodium hydroxide (solid line). Treatment conditions were 2 N NaOH at 60 °C for 15 min.

however, depolymerization occurs prior to opening of the A-ring, and this likely requires oxygen (*19*). In preliminary experiments, we observed that tubes flushed with nitrogen yielded much higher procyanidin levels than those that were not, further validating the idea that degradation beyond depolymerization requires oxygen. Although it is likely that some further degradation is occurring at the conditions that we are using, we have optimized the conditions to produce the highest possible yields of procyanidin oligomers. Exclusion of oxygen allowed us to achieve depolymerization without further degradation.

Other researchers have used the depolymerization principle to synthesize dimeric procyanidins (*25*). They isolated polymeric procyanidins from chokeberry and subjected them to acid catalyzed depolymerization in the presence of the monomeric flavan-3-ols catechin and epicatechin. They were able to synthesize significant levels of procyanidin dimers based on the fact that, upon

depolymerization, a procyanidin extension unit becomes a positively charged carbocation that is capable of reacting with catechin or epicatechin, rather than the typical nucleophiles mentioned above, to form a stable dimer. Given the high levels of dimeric procyanidins observed in this study, it is possible that the carbocation intermediates that are formed are reacting with catechin or epicatechin that is already present in the pomace or released as a terminal unit to form a dimer. Additionally, it has been demonstrated that A-type linkages are resistant to acid catalyzed cleavage, and therefore might also be resistant to base catalyzed cleavage (*21*). This could also explain the high levels of A-type dimers that we observed following alkaline treatment.

Enhanced Extraction of Procyanidins. The increase in total procyanidins after alkaline hydrolysis and the further release of procyanidins from the treated residue indicate that there are bound procyanidins present in cranberry pomace that are released by treatment with alkali. There has been significant interest recently in the presence of bound or unextractable procyanidins in plant materials. There is evidence that many procyanidins are not able to be extracted by conventional methods of extraction, but the means by which they are bound to the cellular material is relatively unknown. It is believed that they may be tightly bound to cell wall material. A series of studies were conducted to determine how procyanidins interact with apple cell wall material (*11*, *26*, *27*). The researchers found that isolated procyanidins bound readily to cell wall carbohydrates, particularly pectin, and binding increased with increasing DP of the procyanidin. Drying also increased the binding of procyanidins to the cell wall. Pinelo et al. (*10*) proposed that interactions between polyphenolics and cell wall material may be hydrophobic interactions between phenols and hydrophobic pockets or hydrogen bonding between hydroxyl groups on phenolics and cell wall polysaccharides. It is also possible, though not proven, that procyanidins may be covalently linked to cell wall components similar to the way ferulic acid is linked to the cell wall of grains (*18*).

Hellström and Matilla (*12*) have developed a method to determine unextractable procyanidins in plant materials by acid-catalyzed depolymerization of the compounds into flavan-3-ols and benzylthioethers using thioacidolysis. They have used this method to determine the amount of unextractable procyanidins in several plant materials including cranberries (*14*). They found that a significant amount of procyanidins in many plant materials were "unextractable." Other researchers have used butanol/HCl with heat to determine the amount of bound procyanidins (*15*, *28*). This method is based on the principle that under heat and acid, procyanidins are converted to cyanidin which can be measured spectrophotometrically. Researchers found that apples, peaches, and nectarines contain higher levels of nonextractable procyanidins than extractable procyanidins (*15*).

These methods are effective in identifying the presence of bound procyanidins; however, problems exist when using these methods for quantification because of the kinetics of the reactions. Thiolysis yields have been reported to be low (34−63%), and this may be due to impurities, thiolysis resistant bonds, or instability of reaction products (*29*). The butanol/HCl assay produces several side reactions which result in lower yields, and not all procyanidins react the same under the reaction conditions (*30*). Additionally, these methods do not preserve the integrity of the procyanidins; therefore, they are unrecoverable.

In contrast, treatment of the residue remaining after conventional extraction of phenolics from cranberry pomace resulted in release of procyanidins in the form of monomers, dimers, and other oligomers, which can be extracted and used in a variety of applications. Although the procyanidins were released as low molecular weight compounds, we do not believe that this indicates that it was the lower oligomers that were tightly bound and unextractable. Rather, given that researchers have shown that it is primarily polymeric procyanidins that bind strongly to cell wall material and therefore resist extraction (*26*), and the fact that procyanidins can be depolymerizied under alkaline conditions, it is likely that treatment with NaOH resulted in release of the polymeric procyanidins in the form of lower oligomers by means of depolymerization. Since "free" procyanidins were removed from the residue before treatment with sodium hydroxide, by comparing the procyanidins released with those obtained by treatment of the whole pomace, we were able to differentiate between procyanidins that were truly bound to the cell matrix and those that were merely depolymerizied from "free" polymeric procyanidins.

The mechanism by which sodium hydroxide enhances the extraction of procyanidins may also lie in its ability to solubilize cell wall material in the cranberry pomace. Dilute sodium hydroxide is commonly used to extract hemicellulose from cell wall material (*31*). It is possible that the solubilization of hemicellulose leads to the release of procyanidins that are entrapped or even esterified to the cell wall, but this needs to be confirmed in a follow-up study.

Application of Alkaline Treatment of Cranberry Pomace. Alkaline treatment of cranberry pomace provides a means of utilizing a waste material to produce valuable procyanidins with potential health benefits that can be used for various nutraceutical purposes. We have demonstrated that sodium hydroxide releases bound procyanidins which can be subsequently recovered for a variety of applications. The most valuable application of this treatment would be to extract anthocyanins and other polyphenolics from the plant material first, and then treat the residue with sodium hydroxide to release the bound procyanidins in a usable form. Although in our experiment, treatment of the residue with NaOH resulted in lower yields than when the whole pomace was treated, a significant amount of procyanidins were further extracted from the residue. The lower yields were because depolymerization of "free" polymeric procyanidins also occurred in the whole pomace, resulting in higher levels of monomers and dimers. The procyanidins extracted from the residue by NaOH treatment represents an estimate of the amount of procyanidins bound to the cell matrix.

Extraction with ethyl acetate provided a means of fractionation of low DP procyanidins from high DP procyanidins and other phenolic compounds. Although aqueous acetone is known to be the most effective solvent to extract procyanidins, the extracts require further purification steps to isolate procyanidins from other polyphenolics (e.g., anthocyanins, flavonols) prior to HPLC analysis. Ethyl acetate proved to be an effective extraction solvent for low DP procyanidins. This could be useful in an industrial application where it might be desired to separate procyanidins based on their molecular weight.

The application of this process is 3-fold. It could be used as a means of estimating the amount of bound procyanidins in many plant materials, thus giving a better idea of the total procyanidins in the product since, although they are unextractable, they may still be biologically important. Second, treatment of procyanidin containing materials with sodium hydroxide could enhance the bioavailability of the compounds since DP1 and DP2 procyanidins were generated and released in the greatest quantities compared to higher oligomers. This is important because several researchers have noted that DP1, DP2, and to a lesser extent DP3 procyanidins are absorbed, whereas higher oligomers are not (*8*). The larger oligomers, however, may still confer health benefits due to their ability to be metabolized by colonic microflora, which

in turn produce smaller molecules such as phenolic acids that may subsequently be absorbed (*32*). Additionally, free procyanidins may be more available for microbial metabolism than those bound within the cell wall. Lastly, treatment with NaOH could be used industrially as means of recovering procyanidins from plant material. Polyphenolics are often recovered from waste materials to be used in dietary supplements or fortification purposes. After anthocyanins, flavonols, and other phenolics have been extracted, the residue could be treated with NaOH, neutralized, and desalted. The released procyanidins could then be extracted for use in a variety of applications.

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