JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Impact of Different Stages of Juice Processing on the Anthocyanin, Flavonol, and Procyanidin Contents of Cranberries

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ABSTRACT: Juice is the most common form in which cranberries are consumed; however there is limited information on the changes of polyphenolic content of the berries during juice processing. This study investigated the effects of three different pretreatments (grinding plus blanching; only grinding; only blanching) for cranberry juice processing on the concentrations of anthocyanins, flavonols, and procyanidins throughout processing. Flavonols and procyanidins were retained in the juice to a greater extent than anthocyanins, and pressing resulted in the most significant losses in polyphenolics due to removal of the seeds and skins. Flavonol aglycones were formed during processing as a result of heat treatment. Drying of cranberry pomace resulted in increased extraction of flavonols and procyanidin oligomers but lower extraction of polymeric procyanidins. The results indicate that cranberry polyphenolics are relatively stable during processing compared to other berries; however, more work is needed to determine their fate during storage of juices.

KEYWORDS: anthocyanins, cranberry, flavonols, juicing, processing, procyanidins

INTRODUCTION

For centuries, the American cranberry (*Vaccinium macrocarpon*) has been recognized for its health-benefiting and medicinal properties. Early American colonists were introduced to the berry by the Native Americans, who used cranberries as a food, a meat preservative, and a treatment for infections and various maladies.[1] Cranberries soon became a common ingredient in sauces, jellies, and, most notably, juices. According to the USDA-NASS, <5% of cranberries are sold fresh, with the remaining 95% processed into juice, sauce, or sweetened dried cranberries.[2]

Berries are widely recognized for their various health-promoting properties including the reduced risks of cancer, cardiovascular disease, and other chronic diseases, which have been attributed to polyphenolic compounds, including anthocyanins, flavonols, and procyanidins.[3,4] The polyphenolic composition of cranberries has been evaluated extensively. There are six major anthocyanins in cranberries, including the arabinosides, glucosides, and galactosides of cyanidin and peonidin. The major flavonols in cranberries are glycosides of quercetin and myricetin with quercetin-3-galactoside predominating. Cranberries contain procyanidins with various degrees of polymerization and are unique in that many of the procyanidins contain at least one A-type linkage, which consists of both $\beta 4 \rightarrow 8$ and $\beta 2 \rightarrow O \rightarrow 7$ bonds between two monomeric units. Most procyanidin containing species have only B-type linkages, which are single $\beta 4 \rightarrow 8$ linkages between monomeric units.

Cranberries are perhaps most recognized for their role in urinary health, specifically their ability to prevent recurrent urinary tract infections.[5] The exact mechanism for cranberries' role in urinary tract prevention has yet to be elucidated; however, recent research has attributed the property to the presence of procyanidins containing A-type linkages. These compounds are believed to prevent uropathogenic bacteria from adhering to the epithelial lining of the urinary tract, thus preventing the onset of an infection.[6]

There has been considerable evidence suggesting that polyphenolic compounds in berries are readily degraded during juice processing. Many studies regarding cranberry juice processing have focused on retention of anthocyanins due to their contribution to the visual quality of juices and overall susceptibility to degradation by heat, light, and enzymatic activity. Anthocyanin retention in cranberry juice is generally <50% due to losses during various stages of processing.[7] Although flavonol retention during cranberry juice processing has never been evaluated, flavonols are generally well retained compared to anthocyanins. However, significant quantities of flavonol aglycones have been reported in processed cranberry products but not in fresh cranberries.[8,9] Likewise, procyanidin retention during cranberry juice processing has not been assessed; however, commercial cranberry juice contains a higher proportion of monomers, dimers, and trimers and fewer higher oligomers compared to fresh berries.[10]

Various factors including temperature, processing duration, and pretreatment of the fruit can affect the retention of polyphenolic compounds in juice. Additionally, freezing of berries prior to juice processing can have a profound effect on processing stability and expression of polyphenolics in the juice. It is important to understand how processing alters the polyphenolic compounds in cranberries so that efforts may be made to retain them and maximize the health benefits of cranberry juice. Therefore, the objectives of this study were to investigate the effects of pretreatments such as grinding and blanching on the polyphenolic content of cranberries throughout juice processing and to determine the effects of drying on the extractability of polyphenolics from cranberry pomace.

Received: January 11, 2011
Revised: March 23, 2011
Accepted: March 25, 2011
Published: March 25, 2011

dx.doi.org/10.1021/jf200149a | *J. Agric. Food Chem.* 2011, 59, 4692–4698

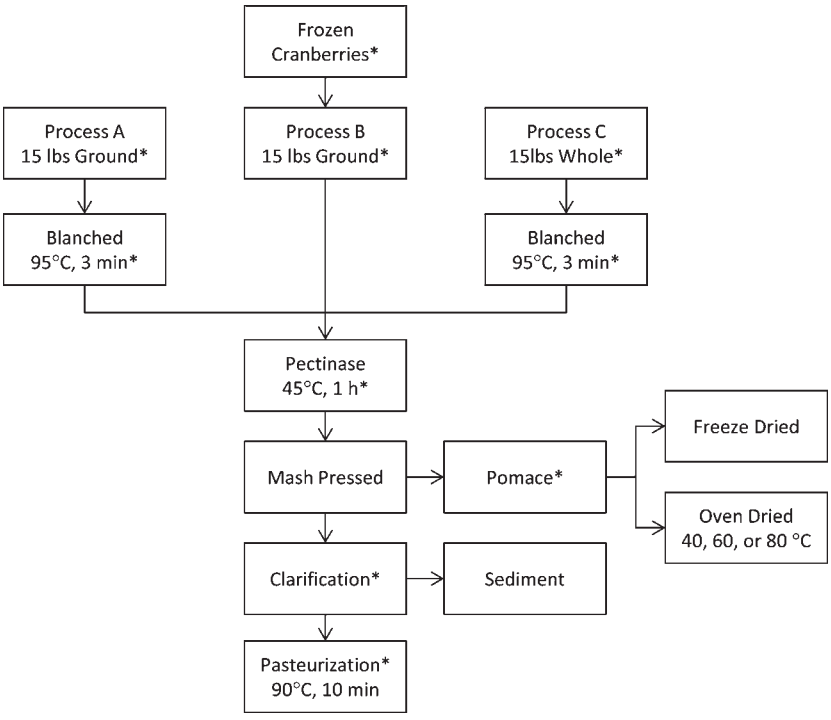

Figure 1. Flowchart of cranberry juice processing with sampling points indicated by asterisks.

MATERIALS AND METHODS

Frozen Berries. Frozen cranberries were obtained from Decas Cranberry Co. (Carver, MA) and stored at −20 °C until processing.

Juice Processing. A juice processing schematic is shown in Figure 1, with sampling points indicated by asterisks. Frozen berries were assigned to one of three pretreatments, which included (A) grinding plus blanching, (B) grinding plus no blanching, and (C) no grinding plus blanching. Coarse grinding of frozen berries was performed for approximately 2 min at 2500 rpm using an industrial food processor (model RSI6 V; Robot Coupe USA, Inc., Jackson, MS). Berries were blanched by heating the mash to 95 °C in a large steam kettle and holding for 3 min. The temperature of the berries was determined by inserting a Doric 400 series thermocouple in the center of the mash. Depectinization was achieved by adding 0.12% w/v of the commercial pectinase enzyme *Crystalzyme* Cran (Valley Research, Inc., South Bend, IN), which has an activity of 40000 APU (apple pomace units), and holding at 45 °C for 1 h or until the mash produced a negative alcohol precipitation test. Depectinization time did not exceed 2 h. Following depectinization, the mash was pressed in a 25 L Enrossi bladder press (Enoagricol Rossi s.r.l., Calzolaro, Italy) at 20 psi to separate the juice from the pomace. The residual moisture content of the pomace after pressing was approximately 73%. Juices were allowed to settle overnight at 4 °C and were then clarified by centrifugation for 10 min at 6000*g* in an Allegra X-22R centrifuge (Beckman Coulter, Brea, CA). The clarified juice was then filled into 5 mL screw-top glass tubes and pasteurized by placing in a 90 °C water bath for 10 min and then cooling in an ice bath. The soluble solids contents of the juices were determined to be 11.2, 9.0, and 10.0 °Brix for process A, B, and C juices, respectively. The pH of the juices was 2.6.

Drying of Pomace. Pomace resulting from pressing of juices was dried by freeze-drying using a VirTis Genesis freeze-dryer (Gardiner, NY) or oven-dried using a forced-air oven in 250 g quantities each. Oven-drying was performed at three different temperatures (40, 60, or 80 °C), and samples were allowed to dry until the pomace reached a moisture content of 4−5%. Drying times for 40, 60, and 80 °C were approximately 4, 8, and 16 h, respectively.

Extraction of Polyphenolics. Frozen cranberries and samples from each step along the processing method were homogenized with 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v) using a Euro Turrax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, OH) and filtered through Miracloth (Calbiochem, La Jolla, CA). The residue was collected, the extraction repeated two more times, and the volume of the extracts adjusted to 100 mL with extraction solvent. Juice samples were analyzed directly and required no extraction.

HPLC Analysis of Anthocyanins. Extracts (8 mL) were dried using a SpeedVac concentrator (ThermoSavant, Holbrook, NY) and resuspended in 1 mL of 3% formic acid in water. Individual anthocyanins were separated by reverse phase HPLC according to the method described by Cho et al.[11] Anthocyanin glycosides were quantified as corresponding anthocyanin glucosides using external calibration curves of a mixture of anthocyanin glucoside standards purchased from Polyphenols Laboratories (Sandnes, Norway).

HPLC Analysis of Flavonols. Extracts (8 mL) were dried using a SpeedVac concentrator and resuspended in 1 mL of 50% methanol in water. Individual flavonols were separated by reverse phase HPLC according to the method described by Schieber et al.[12] Flavonol glycosides were quantified as rutin equivalents and aglycones as either quercetin or myricetin using external calibration curves of standards purchased from Sigma Chemical Co. (St. Louis, MO).

HPLC Analysis of Procyanidins. Acetone was evaporated from extracts (20 mL), and the resulting aqueous fraction was subjected to solid-phase extraction according to the method described by Gu et al.[13] to remove interfering sugars and other phenolic compounds. Resulting extracts were evaporated to dryness using a SpeedVac concentrator, resuspended in 2 mL of extraction solvent, and filtered through 0.45 μm filters. Procyanidins were separated by the method of Hammerstone et al.[14] and quantifed using external calibration curves of a mixture of procyanidin standards (DP1−DP6) isolated from cocoa and obtained from Mars, Inc. (Hackettstown, NJ).[15] A-type procyanidins were quantified as B-type

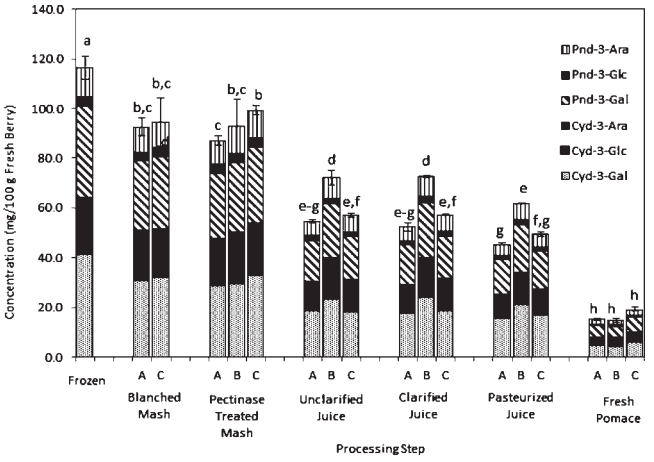

Figure 2. Concentration of anthocyanins throughout cranberry juice processing. Bars represent standard errors of total anthocyanins ($n = 3$). Bars with different letters indicate that values are statistically different ($p < 0.05$). Pretreatments are as follows: Process A berries were ground and blanched. Process B berries were ground only. Process C berries were blanched only. Frozen sample represents process B ground sample.

equivalents. Polymeric procyanidins (DP > 10) were quantified by extrapolation of a slope versus degree of polymerization curve.

Calculations. To account for dilution and concentration effects, anthocyanin, flavonol, and procyanidin concentrations were converted to original berry weight so that all samples could be compared on an equivalent basis. This was done using the equation

$$C_{berry} = C_{product} \times R$$

where C_{berry} = concentration on original berry weight basis, R = mass of the product divided by mass of original berry, and $C_{product}$ = concentration in the product.

Statistical Analysis. Three samples were taken at each sampling point along the juicing process. Data are expressed as the mean ± standard error. The effects of processing on anthocyanin, flavonol, and procyanidin concentrations were determined by one-way analysis of variance (ANOVA) using JMP 8.0 (Cary, NC). Differences between means were determined using Student's t test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Processing Effects on Anthocyanins. Six anthocyanin glycosides were identified and quantified in the cranberries as previously described.[16] Changes in anthocyanin content were evaluated throughout juice processing, and the results are presented in Figure 2. Blanching resulted in significant losses of total anthocyanins. It appeared that the type of sugar attached affected the stability of the anthocyanin during blanching. The most stable were the glucosides of cyanidin and peonidin with 100% retention after blanching, whereas their respective galactosides were the least stable, with only 76−77% retention after blanching. Several studies have reported that anthocyanin stability is dependent upon the type of sugar attached and not the aglycone, and glucosides are more stable than galactosides, which are in turn more stable than arabinosides.[17,18] However, we found that during blanching, the arabinosides were more stable than the galactosides. No further losses of total anthocyanins were observed during depectinization.

The greatest losses in total anthocyanins were observed during pressing, presumably due to the exclusion of seeds and skins in the pomace, which retained 13−17% of the total anthocyanins.

Table 1. Percent Retention[a] of Polyphenolics in Cranberry Juice and Pomace

	anthocyanins	flavonols	procyanidins
Process A			
pasteurized juice	39.0	57.4	48.9
pomace	13.1	35.6	39.4
Process B			
pasteurized juice	53.1	67.2	54.7
pomace	12.8	27.7	40.4
Process C			
pasteurized juice	42.4	57.0	57.4
pomace	16.6	33.0	40.5

[a] Percent retention of pasteurized juice and fresh pomace compared to frozen cranberries.

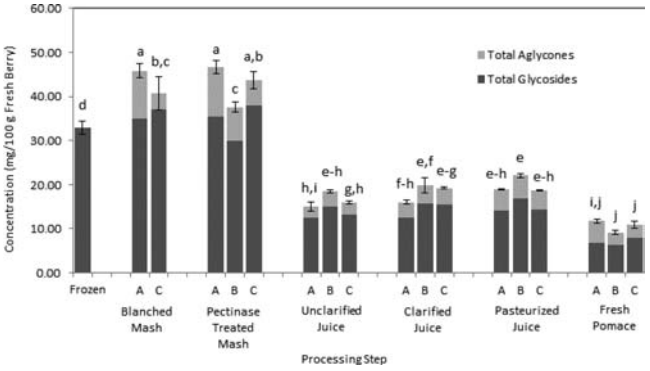

Figure 3. Concentration of flavonols throughout cranberry juice processing. Bars represent standard errors of total flavonols ($n = 3$). Bars with different letters indicate that values are statistically different ($p < 0.05$). Pretreatments are as follows: Process A berries were ground and blanched. Process B berries were ground only. Process C berries were blanched only. Frozen sample represents process B ground sample.

Interestingly, a greater amount of anthocyanins was expressed in the unclarified juice from the berries that were ground but not blanched (process B). Immediate clarification by centrifugation did not result in anthocyanin loss due to sedimentation. Slight losses were observed due to pasteurization only in berries pretreated using process B. In contrast to blanching, but consistent with previous studies, the order of anthocyanin stability during pasteurization was (from most to least stable) glucosides > galactosides > arabinosides. Total recovery of anthocyanins in pasteurized juices ranged from 39 to 53%, which is consistent with a previous study on cranberries.[19] Relative retentions of anthocyanins in pasteurized juices and fresh pomaces compared to frozen cranberries are presented in Table 1.

These findings suggest that grinding of the fruit as the first step in juice processing enhances expression of anthocyanins into the juice, and blanching of the berries to inactivate endogenous enzymes, such as polyphenol oxidase and glycosidases, does not necessarily provide additional protection against anthocyanin degradation. In this case, blanching reduced expression of anthocyanins into the juice, likely because it caused moisture loss in the berries, which resulted in decreased mass transfer of

anthocyanins into the juice. This is in stark contrast to studies involving blueberry processing that found blanching to significantly improve anthocyanin retention in processed products.[20] Anthocyanin retention in red raspberry juice, however, was unaffected by blanching.[20] This is likely due to the difference in activities of endogenous enzymes in different berries. Polyphenol oxidase in blueberries is responsible for significant losses in anthocyanins during processing and must be inactivated by blanching to prevent these losses.[21] This enzyme is apparently less active in cranberries due to either their naturally low pH or insufficient quantities of enzyme or its substrate, simple phenols.

Processing Effects on Flavonols. Changes in total flavonol aglycones and glycosides were followed throughout processing, and the results are presented in Figure 3. Blanching resulted in a significant increase in total flavonols, and no change was observed after blanched berries were depectinized. Compared to frozen berries, an increase in total flavonols in pectinase-treated berries that were not blanched (process B) was also observed, but the increase was not as pronounced as berries that were blanched. This indicates that cranberry flavonols are relatively heat stable compared to anthocyanins, and blanching and grinding facilitated their extraction by membrane disruption. Pressing resulted in significant losses of flavonols due to the exclusion of seeds and skins in the pomace, which retained 28−36% of the total flavonols present before pressing. Additional losses could be attributed to binding of the flavonols to cell wall material, which may have caused them to be unextractable. Clarification and pasteurization had no effect on total flavonol concentration of the juices. Relative retentions of total flavonols in pasteurized juices and fresh pomaces compared to frozen cranberries are presented in Table 1. Individual flavonol retention appeared to be dependent upon the type of sugar attached rather than the aglycone. Myricetin arabinoside and quercetin arabinofuranoside were considerably less stable than the other glycosides, with only 22 and 24% retained in pasteurized juices, respectively. This is consistent with a study that found quercetin arabinoside to be the least stable flavonol glycoside during storage of apple juice.[22] Interestingly, quercetin arabinopyranoside was also present in the frozen cranberries and showed remarkably greater stability (55%) than the furanoside.

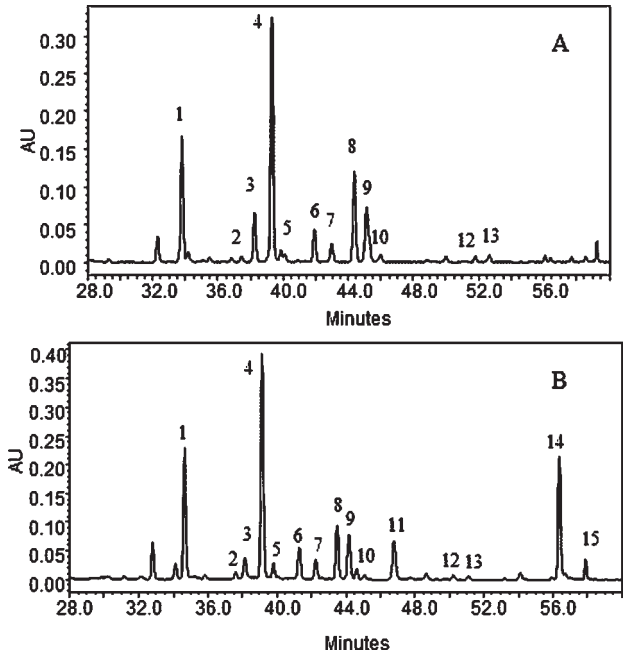

Figure 4. HPLC chromatograms of flavonols in frozen cranberries (A) and blanched cranberries (B) detected at 360 nm. See Table 2 for peak identification.

Changes in the composition of flavonols were also observed during juice processing. HPLC chromatograms of flavonols in frozen and blanched cranberries are shown in Figure 4, and peak identification is shown in Table 2. Flavonol aglycones, myricetin and quercetin, were observed after blanching, but not in frozen berries, and their quantities increased as processing progressed. This was coupled with slight decreases in the amount of flavonol glycosides, indicating deglycosylation of the glycosides into aglycones as a result of processing. In pasteurized juices, flavonol aglycones comprised 24−25% of the total flavonols. Additionally, the pomace contained a higher percentage of aglycones (26−43%) than did the juices, indicating that aglycones are not as readily expressed in juices because they are less polar than their

Table 2. Peak Identification and Mass Spectral Data of Flavonols Detected in Cranberries by HPLC-ESI-MS

		mass to charge ratios (m/z)	
peak	flavonol	$[M - H]^-$	fragments
1	myricetin 3-galactoside	479	316 [M − galactose]
2	myricetin 3-xyloside	433	316 [M − xylose]
3	myricetin 3-arabinoside	449	316 [M − arabinose]
4	quercetin 3-galactoside	463	300 [M − galactose]
5	quercetin 3-glucoside	463	300 [M − glucose]
6	quercetin 3-xyloside	433	300 [M − xylose]
7	quercetin 3-arabinopyranoside	433	300 [M − arabinose]
8	quercetin 3-arabinofuranoside	433	300 [M − arabinose]
9	quercetin 3-rhamnoside	447	300 [M − rhamnose]
10	methoxyquercetin 3-galactoside	477	315 [M − galactose], 300 [M − galactose + methoxy]
11	myricetin	317	(myricetin)
12	quercetin 3-glucuronide	477	301 [M − glucuronic acid]
13	quercetin 3-coumaroylgalacoside	609	463 [M − galactose], 300 [M − coumaric acid + galactose]
14	quercetin	300	(quercetin)
15	quercetin 3-benzoylgalactoside	567	300 [M − galactose + benzoic acid]

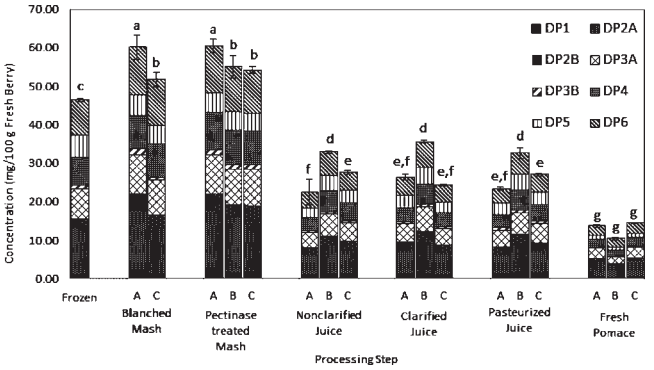

Figure 5. Concentration of procyanidin oligomers throughout cranberry juice processing. Bars represent standard errors of total procyanidins ($n = 3$). Bars with different letters indicate that values are statistically different ($p < 0.05$). Pretreatments are as follows: Process A berries were ground and blanched. Process B berries were ground only. Process C berries were blanched only. Frozen sample represents process B ground sample.

glycosidic counterparts. Other researchers have also noted significant quantities of flavonol aglycones in processed cranberry products but not in fresh berries.[8] It was previously hypothesized that the presence of flavonol aglycones in cranberry pomace was the result of deglycosylating side activities of pectinase enzymes used for juice processing;[16] however, this was unlikely the case because the aglycones were found immediately after blanching before addition of the enzyme. Therefore, because cranberries have a very low pH (2.6) compared to other berries, we believe that it is a combination of heat and low pH that causes deglycosylation of flavonol glycosides. To test this hypothesis, we treated solutions of quercetin-3-glucoside under conditions that mimicked blanching and found that in the solution that was adjusted to pH 2.6 and blanched, quercetin levels increased by 15%. However, in the solution that was blanched at their original pH (4.9), quercetin levels decreased by 6%. This is the first demonstration that a combination of heat and low pH in cranberries can result in the formation of significant quantities of flavonol aglycones. Increases in quercetin have also been observed in cranberry jam, however, not to the extent that we found in this study.[9]

Processing Effects on Procyanidins. Procyanidins of DP1−DP6 as well as polymeric procyanidins (DP > 10) were identified in the cranberries as previously described.[16] Changes in procyanidin content and composition were evaluated throughout processing, and the results are presented in Figure 5 and Table 3. Blanching resulted in an increase in both total procyanidin oligomers (DP1−DP6) and polymers (DP > 10). No further changes in procyanidin concentrations were observed as a result of depectinization. Berries pretreated by process B retained higher levels of polymeric procyanidins after depectinization than those that were blanched, indicating that blanching caused a decrease in polymeric procyanidins. This could be the result of depolymerization of polymeric compounds to smaller ones or binding of polymers to the cell wall material, which may render them unextractable. Polymeric procyanidins have been shown to readily bind to the cell wall.[23] Relative retentions of total procyanidins in pasteurized juices and fresh pomaces compared to frozen cranberries are presented in Table 1. Pressing resulted in significant losses of procyanidins as a result of seed and skin exclusion in the pomace, which retained 22−31% of the oligomers, 43−52% of the polymeric procyanidins, and approximately 40% of the total procyanidins. Interestingly,

Table 3. Concentration of Procyanidin Polymers during Cranberry Juice Processing

processing step	pretreatment[a]	polymer concentration[b] (mg/100 g of frozen berry)	% polymer
frozen		206.2 ± 9.3 c	81.7
blanched	A	251.8 ± 8.4 b	80.7
	C	245.3 ± 18.5 b	82.6
enzyme-treated mash	A	261.4 ± 20.9 b	81.2
	B	319.6 ± 11.5 a	85.3
	C	172.7 ± 8.6 d	76.1
unclarified juice	A	104.2 ± 17.7 e−h	82.3
	B	103.5 ± 8.2 e−i	76.0
	C	100.7 ± 4.3 f−j	78.5
clarified juice	A	107.7 ± 3.6 e−g	80.4
	B	74.0 ± 10.9 jk	67.5
	C	86.2 ± 4.5 g−k	78.0
pasteurized juice	A	76.1 ± 4.0 h−k	76.5
	B	69.4 ± 2.7 k	68.0
	C	75.3 ± 7.2 i−k	73.6
pomace	A	109.7 ± 1.7 e−g	88.9
	B	127.7 ± 3.9 ef	92.5
	C	130.4 ± 0.5 e	90.0

[a] Pretreatments included (A) grinding plus blanching, (B) grinding with no blanching, (C) no grinding plus blanching. [b] Values represent means ± standard error. Values within the column followed by the same letters are not significantly different ($p > 0.05$).

juices resulting from berries that were pretreated by process B (grinding, no blanching) had higher levels of procyanidin oligomers than did the other juices. Clarification and pasteurization had no effect on the concentration of procyanidin oligomers in the juices. The pomaces contained a higher percentage of their procyanidins as polymeric compounds than did the other products, which suggests that the lower oligomers are more readily expressed in the juice than polymeric procyanidins.

Effect of Drying on Polyphenolics in Pomace. Polyphenolics were extracted from pomaces that were either freeze-dried or oven-dried (40, 60, or 80 °C), and these results are presented in Table 4. Drying had no effect on total anthocyanins in the pomace. This is consistent with a study on grape pomace, in which no losses in total extractable polyphenols or antioxidant activity were observed at temperatures of <100 °C compared to freeze-dried pomace.[24] Freeze-drying resulted in increased extractability of flavonol glycosides, but they were unaffected by oven-drying. Oven-drying at 60 and 80 °C, however, did increase the amount of flavonol aglycones in the pomace. This was also observed during juice processing and is likely due to heat-induced deglycosylation of flavonol glycosides at the naturally low pH of cranberries. Drying also increased the amount of procyanidin oligomers extracted from the pomace, with the

Table 4. Concentration of Polyphenolics in Fresh, Freeze-Dried, and Oven-Dried Cranberry Pomace

	concentration[a] (mg/100 g DW)				
		flavonols		procyanidins	
pomace type	anthocyanins	glycosides	aglycones	oligomers	polymers
fresh	362.5 ± 36.6 a	160.0 ± 20.2 b	119.0 ± 10.1 b	332.7 ± 18.9 d	2629.1 ± 85.0 a
freeze-dried	366.1 ± 8.6 a	191.1 ± 1.6 a	121.4 ± 0.7 b	556.5 ± 12.6 a	1860.5 ± 59.5 bc
oven-dried (40 °C)	380.7 ± 18.1 a	152.2 ± 7.0 b	137.0 ± 9.8 b	498.1 ± 0.8 b	1954.4 ± 36.2 b
oven-dried (60 °C)	342.9 ± 7.8 a	137.9 ± 2.4 b	163.6 ± 3.4 a	462.9 ± 8.0 c	1771.8 ± 32.0 c
oven-dried (80 °C)	365.9 ± 11.5 a	149.7 ± 0.8 b	161.4 ± 6.5 a	503.0 ± 4.8 b	1939.8 ± 44.1 bc

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

freeze-dried pomace containing the highest amount. In contrast, polymeric procyanidins were lower in dried pomace when compared to fresh. This could be due to depolymerization of polymeric procyanidins or increased binding of the polymeric compounds to the cell wall matrix during drying. The increase in flavonols and procyanidin oligomers after drying indicates that these compounds were ineffectively extracted from the fresh pomace, and drying aided in extraction. Drying has been shown to decrease cell wall porosity, resulting in collapse of the cell wall structure, which decreases the affinity between cell wall polysaccharides and polyphenolics.[25] In this study, polymeric procyanidins were not affected by the decrease in porosity, as their binding seemed to increase upon drying. Polymers have a higher affinity for cellular material than do lower molecular weight compounds due to the presence of multiple binding sites on large molecules.[23,25]

In summary, flavonols and procyanidins were generally more stable than anthocyanins during cranberry juice processing. The most significant losses of polyphenolics were observed during pressing as a result of exclusion of the seeds and skins. Blanching of the fruit prior to processing did not improve polyphenolic retention; however, grinding of the fruit appeared to facilitate expression of polyphenolics into the juice. Increases in flavonol aglycones were observed during juice processing as a result of deglycosylation of flavonol glycosides by heating. On the basis of these findings, it is recommended that frozen fruit be ground prior to further juice processing to maximize the polyphenolic content of cranberry juice; however, a blanching step may not be necessary to inactivate polyphenol oxidase in cranberry fruit due to low pH or low enzyme activity. Drying of cranberry pomace did not affect anthocyanin concentrations; however, extraction of flavonols and procyanidin oligomers was improved by drying, likely as a result of a decrease in porosity of the cell wall. Our results indicate that cranberry polyphenolics are relatively stable during juice processing compared to other berries; however, more work is needed to determine their fate during storage.

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ACKNOWLEDGMENT

We thank Decas Cranberry Co., Inc., for providing the frozen cranberries and pectinase used in this study.

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