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Polyphenolic Composition and Antioxidant Capacity of Extruded Cranberry Pomace†

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Cranberry pomace was mixed with corn starch in various ratios (30:70, 40:60, 50:50 pomace/corn starch DW) and extruded using a twin-screw extruder at three temperatures (150, 170, 190 °C) and two screw speeds (150, 200 rpm). Changes in the anthocyanin, flavonol, and procyanidin contents due to extrusion were determined by HPLC. Antioxidant capacity of the extrudates was determined using oxygen radical absorbance capacity (ORAC). Anthocyanin retention was dependent upon barrel temperature and percent pomace. The highest retention was observed at 150 °C and 30% pomace. Flavonols increased by 30−34% upon extrusion compared to an unextruded control. ORAC values increased upon extrusion at 170 and 190 °C. An increase in DP1 and DP2 procyanidins was also observed; however, a decrease was observed in DP4−DP9 oligomers. These data suggest that extrusion alters the polyphenolic distribution of cranberry pomace and has application in the nutraceutical industry as a means of improving the functionality of this coproduct.

KEYWORDS: Anthocyanins; cranberry; extrusion; flavonols; pomace; procyanidins

INTRODUCTION

Cranberry pomace is the byproduct of the cranberry-processing industry and is composed of skin, seeds, and stems, which remain after the fruit has been pressed for juice or prepared for canning. Applications for cranberry pomace are limited. Its low protein content makes it unsuitable for animal feeds, and its low pH presents problems when it is disposed of in the soil (*1*).

Plant foods have been recognized for their health benefits including reduced risk of cancer and cardiovascular disease. Cranberries in particular are recognized for their ability to prevent urinary tract infections (*2*). These benefits have been attributed to the presence of polyphenolic compounds such as anthocyanins, flavonols, and procyanidins (*3*, *4*). These compounds are found primarily in the seeds and skins of the fruit; thus, many are retained in the pomace. Cranberry pomace, therefore, should be explored as a source of polyphenolic compounds.

Anthocyanins are pigmented compounds present in the epidermal tissues of fruits and vegetables (*5*). There are six major anthocyanins found in cranberries, and they include the glucosides, galactosides, and arabinosides of cyanidin and peonidin (*6*). Minor anthocyanins that might be present in some cranberry varieties include glycosides of malvidin, petunidin, delphinidin, and pelargonidin. Flavonols are another class of polyphenolic compounds present in cranberries. Flavonols impart bitter and astringent flavors to fruits and vegetables (*7*). Cranberries contain relatively high levels of glycosides and other derivatives of the flavonol quercetin, compared to other fruits. As many as 22 different flavonol glycosides have been identified in cranberries including primarily derivatives of quercetin and myricetin and two kaempferol derivatives (*8*).

Procyanidins are a class of polymeric compounds composed of flavan-3-ol monomeric units. These monomeric units may be linked in two ways. The most common linkages are called B-type and are $\beta4 \rightarrow 6$ or $\beta4 \rightarrow 8$. The less common A-type linkage contains both a $\beta4 \rightarrow 8$ and a $\beta2 \rightarrow O \rightarrow 7$ linkage. Cranberries are unique in that they contain many procyanidins with A-type linkages (*9*). It is the A-type linkages in cranberry procyanidins that are believed to be responsible for cranberries' ability to prevent urinary tract infections by inhibiting bacteria from adhering to the epithelial lining of the urinary tract (*10*).

A majority of the procyanidins found in cranberries have a high degree of polymerization (DP) (*11*). Donovan and others (*12*) and Gonthier and others (*13*) have shown that although high DP procyanidins are good antioxidants, they are poorly absorbed relative to their monomeric subunits. Although typically present in smaller quantities, monomers, and even dimers and trimers, are better absorbed and maintain similar health benefits (*14*, *15*).

Extrusion has become a popular food-processing technique especially in the cereal and snack food industry. It is considered to be a high-temperature−short-time (HTST) processing method and is capable of preserving desirable food components and destroying microorganisms. The end products are typically low moisture, which allows them to be shelf-stable (*16*). Extrusion may include several different operations; however, the simplest definition is the forcing of a dough-like material through a restriction or die. Other functions of an extruder can include, but are not limited to, homogenization, shearing, thermal

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cooking, gelatinization, and texture alteration (*17*). Extrusion presents mechanical stress in the form of heat and shear to the substrates, which may alter physical as well as chemical characteristics of the product. Because extrusion is a common food-processing practice, many studies have been done to determine its effects on the nutritional aspects of food including flavonoids, which are known to be heat sensitive; these effects may be beneficial or detrimental.

The objectives of this research were to determine the effect of extrusion processing on the anthocyanin, flavonol, and procyanidin composition of cranberry pomace mixed with corn starch. Additionally, the effect of extrusion on the antioxidant capacity of cranberry pomace was evaluated. This research aims to investigate an alternative use of cranberry pomace by improving its functionality.

MATERIALS AND METHODS

Sample Preparation. Dried cranberry pomace (Decas Cranberry Co., Carver, MA) was stored at −20 °C prior to extrusion. For extrusion, the pomace was mixed with corn starch (National Starch, Bridgewater, NJ) in ratios of 30:70, 40:60, and 50:50 cranberry pomace/corn starch on a dry weight basis and mixed using an industrial kitchen mixer (Hobart, Troy, OH). Corn starch served as facilitator for extrusion and allowed extrudates to expand upon exiting the die. Water was added to the mixtures to bring the moisture content to 30%, and they were mixed again. Mixtures were stored at 4 °C overnight prior to extrusion to allow for moisture equilibration.

Extrusion. Cranberry pomace/corn starch mixtures were extruded using a PolyLab-scale Rheomix twin-screw extruder (Thermo Haake, Karlsruhe, Germany) equipped with a 6 mm capillary rod die. The barrel temperature of zone 1 was set at 90 °C for all extrusion runs to prevent moisture loss upon introduction of the mixture to the extruder. The mixtures were continuously fed manually into the extruder. Extrudates were collected with extruder barrel and die temperatures of 150, 170, and 190 °C and screw speeds of 150 and 200 rpm. Extrudates were allowed to cool, placed in sealed bags, and stored at −20 °C until analysis. The moisture content of the extrudates was determined according to the AOAC oven method.

Extraction of Polyphenolics from Extrudates. Extrudates were ground using a commercial coffee grinder prior to extraction of polyphenolics. Ground extrudates (1 g) were mixed with 20 mL of acetone/water/acetic acid (70:29.5:0.5 v/v/v), homogenized with a T18 Basic Ultra-Turrax homogenizer (IKA Works, Wilmington, NC), and filtered through Miracloth. Two more extractions were performed, the extracts were pooled, and the volume was adjusted to 100 mL with extraction solvent.

Sephadex LH-20 Isolation of Procyanidins. Procyanidins were isolated from the extracts according to the method described by Gu et al. (*18*). This was done to prevent the interference of other compounds, such as anthocyanins, sugars, and flavonols, during HPLC analysis. Briefly, 10 mL of extract was concentrated using a SpeedVac vacuum concentrator (ThermoSavant, Holbrook, NY) to remove the acetone. The remaining aqueous extract (3 mL) was loaded onto a manually packed 6 by 1.5 cm column containing 3 g of Sephadex LH-20 (hydrated for at least 4 h). The column was attached to a Sep-Pak vacuum manifold (Waters Corp., Milford, MA) and vacuum pump. The column was flushed with 40 mL of 30% methanol, and this fraction was discarded. The procyanidins were then eluted from the column with 80 mL of 70% acetone. This fraction was collected for HPLC analysis.

HPLC Analysis of Procyanidins. The aqueous acetone fractions resulting from the Sephadex LH-20 isolation of procyanidins were evaporated to dryness using a SpeedVac vacuum concentrator, resuspended in 2 mL acetone/water/acetic acid (70:29.5:0.5), and filtered through 0.45 μm filters prior to HPLC analysis. Procyanidins were separated according to the method described by Kelm et al. (*19*) with slight modifications. A 5 μm, 250 by 4.6 mm, Develosil diol 100A column (Phenomenex, Torrence, CA) was attached to a Waters Alliance 2690 HPLC (Waters Corp., Milford, MA) equipped with a model 474 scanning fluorescence detector. The mobile phase consisted of a binary gradient of 98:2 v/v acetonitrile/acetic acid (A) and 95:3:2 v/v/v methanol/water/acetic acid (B). The flow rate was set at 0.8 mL/min, and the gradient proceeded as follows: 0−35 min, 0−40% B; 35−49 min, 40% B isocratic; 49−50 min, 40−100% B; 50−57, 100% B isocratic; 57−60 min, 100−7% B; 60−70 min, 7% B isocratic. Procyanidins were monitored using fluorescence detection (ex 276 nm, em 316 nm), and monomers through nonamers were quantified on the basis of an external calibration curve consisting of a mixture of procyanidins standards (DP1−DP9) previously purified from cocoa (*19*). Results were expressed on a DW basis.

HPLC Analysis of Anthocyanins. Eight milliliters of polyphenolic extracts was evaporated to dryness using a SpeedVac vacuum concentrator, resuspended in 1 mL of 3% formic acid, and filtered through 0.45 μm filters prior to HPLC analysis. A 4.6 by 250 mm Symmetry C_{18} column (Waters Corp.) was attached to a Waters Alliance Delta 600 HPLC equipped with a model 2996 photodiode array detector. Separations were conducted on the basis of the method described by Cho et al. (*20*). The mobile phase consisted of a linear gradient of 5% formic acid (A) and methanol (B). The flow rate was set at 1.0 mL/min of 2−60% B for 60 min. Anthocyanins were detected at 520 nm and quantified using external calibration curves of anthocyanin glucoside standards obtained from Polyphenols (Sandnes, Norway). Results were expressed on a DW basis.

HPLC Analysis of Flavonols. Eight milliliters of polyphenolic extracts was evaporated to dryness using a SpeedVac vacuum concentrator, resuspended in 1 mL of 50% methanol, and filtered through 0.45 μm filters prior to HPLC analysis. A 5 μm, 4.6 by 250 mm, Aqua C_{18} column (Phenomenex) was attached to a Waters HPLC equipped with a model 996 photodiode array detector. The mobile phase consisted of a gradient of 2% acetic acid (A) and 0.5% acetic acid in 1:1 v/v water/acetonitrile (B). The flow rate was set at 1.0 mL/min, and the gradient proceeded as follows: 0−50 min, 10−55% B; 50−60 min, 55−100% B; 60−65 min, 100−10% B; 65−70 min, 10% B isocratic. Flavonols were detected at 360 nm and quantified using external calibration curves of quercetin or myricetin standards (Sigma Chemical Co., St. Louis, MO). Results were expressed on a DW basis.

Oxygen Radical Absorbance Capacity (ORAC). The antioxidant capacities of cranberry pomace and extrudates were evaluated using the method described by Prior and others (*21*). The method was carried out using a FLUOstar Optima microplate reader (BMG Labtechnologies, Durham, NC). Extracts were diluted 100-fold with phosphate buffer (7 mM, pH 7) prior to analysis. Clear 48-well (590 μL each) Falcon plates (VWR, St. Louis, MO) were used. Forty microliters of diluted sample, Trolox standards (6.25, 12.5, 25, 50 μM), and a blank solution (phosphate buffer) were added to each well. The instrument automatically injected 400 μL of fluorescein (0.108 μM) followed by 150 μL of 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) (31.6 mM) to each well. Fluorescence was detected at 485 nm (excitation) and 520 nm (emission) after the addition of fluorescein and AAPH and every 192 s thereafter for 112 min to allow for a 95% loss of fluorescence. Results were calculated on the basis of differences between the blank, sample, and standard Trolox curves. A standard curve was generated by plotting the concentrations of Trolox against the area under each curve. ORAC values were calculated using the regression equation obtained and expressed as micromoles of Trolox equivalents per gram of DW.

Experimental Design and Statistical Analysis. Treatments were applied in a split−split plot randomized block design with extruder barrel temperature as the whole plot, percent pomace as the split-plot, and screw speed as the split−split plot. As previously mentioned, there were 3 levels of extruder barrel temperate, 3 levels of percent pomace, and 2 levels of screw speed, resulting in 18 conditions. Each condition was run in triplicate to give a total of 54 extrusion runs. Levels of each factor within each plot were randomized.

Analysis of variance and separation of the means were carried out using the PROC MIXED procedure of SAS (SAS V.9.1, SAS Institute, Cary, NC). When effects were significant, comparisons were performed using protected LSD to determine differences among treatments. Additionally, to determine if a treatment was different from the control, confidence intervals ($\alpha = 0.05$) of the least-squares means were used. If the confidence interval for the means included 1, the treatment was considered not to be different from the control.

RESULTS AND DISCUSSION

Anthocyanins. The anthocyanin composition of the cranberry pomace is presented in **Table 1**. Consistent with previous reports

Table 1. Polyphenolic Composition and Content of Cranberry Pomace

compound	concentration[a] (mg/100 g of DW)
Anthocyanins	
cyanidin 3-galactoside	13.2 ± 0.2
cyanidin 3-glucoside	4.5 ± 0.2
cyanidin 3-arabinoside	49.6 ± 6.8
peonidin 3-galactoside	20.1 ± 0.5
peonidin 3-glucoside	7.4 ± 0.3
peonidin 3-arabinoside	26.6 ± 0.5
total	121.4 ± 5.9
Flavonols	
myricetin 3-xyloside	1.5 ± 0.3
myricetin 3-arabinoside	1.8 ± 0.1
quercetin 3-galactoside	12.8 ± 3.6
quercetin 3-xyloside	5.5 ± 0.3
quercetin 3-arabinopyranoside	15.2 ± 3.6
quercetin 3-arabinofuranoside	16.7 ± 3.5
quercetin 3-rhamnoside	18.5 ± 3.4
myricetin	55.6 ± 2.6
methoxyquercetin 3-xyloside	11.4 ± 3.7
quercetin 3-coumaroyl galactoside	2.3 ± 0.3
unidentified	12.1 ± 3.5
quercetin	146.2 ± 22.7
quercetin 3-benzoyl galactoside	27.5 ± 3.4
total	358.4 ± 16.3
Procyanidins	
monomer (DP1)	5.1 ± 0.0
dimer (DP2)	52.7 ± 1.7
trimer (DP3)	30.7 ± 1.4
tetramer (DP4)	16.1 ± 1.3
pentamer (DP5)	22.7 ± 1.2
hexamer (DP6)	25.6 ± 1.2
heptamer (DP7)	16.6 ± 1.2
octamer (DP8)	16.1 ± 2.9
nonomer (DP9)	13.2 ± 1.1
total	186.5 ± 8.8

[a] Mean values ± standard deviation (n = 3).

on fresh cranberries, six anthocyanins were identified in cranberry pomace, including the glucosides, galactosides, and arabinosides of cyanidin and peonidin (*6*). Significant losses of total anthocyanins were observed for all extrusion conditions, and anthocyanin loss was dependent upon extruder barrel temperature and percent pomace. Differences in screw speed were not significant, and there were no significant interactions among the factors. **Figure 1** shows the change in anthocyanin content for each barrel temperature. At 150 °C, 46% of anthocyanins were lost. Losses were greater with higher barrel temperatures of 170 and 190 °C, with 61 and 64% losses, respectively. Degradation was observed for all individual anthocyanins; however, cyanidin 3-arabinoside and peonidin 3-arabinoside were the most affected. The anthocyanin retention observed at 150 °C was much higher than anthocyanin retentions reported for extruded corn breakfast cereals containing blueberry, cranberry, raspberry, and grape powders (10%) (*22*) and for extruded blueberry−corn cereals (32%) (*23*). This discrepancy was likely due to differences in moisture content, amount of anthocyanin-containing material, and extrusion conditions.

Anthocyanin losses were also dependent upon the level of pomace in the extruded mixture (**Figure 2**). The least loss in anthocyanins was observed in the mixture containing only

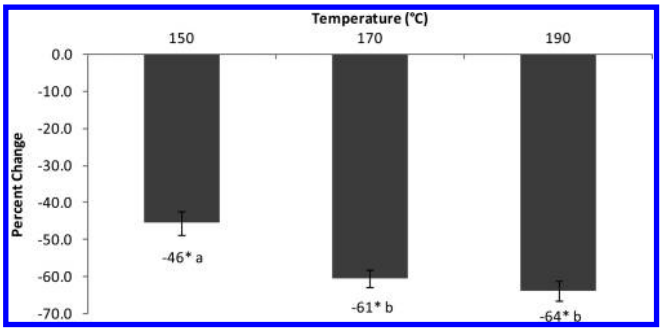

Figure 1. Change in total anthocyanin content of cranberry pomace extruded at different temperatures. Values with different letters are significantly different (p < 0.05). An asterisk indicates values that are significantly different (p < 0.05) from an unextruded control.

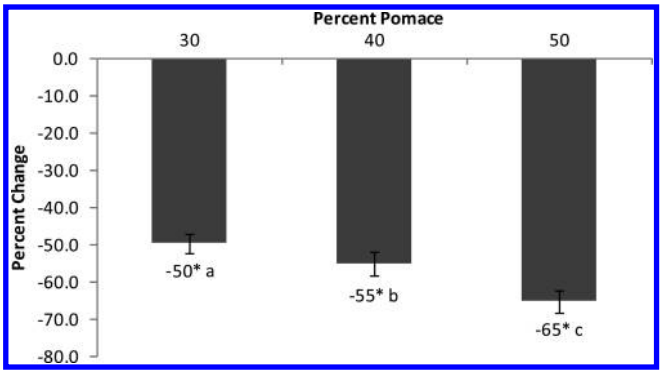

Figure 2. Change in total anthocyanin content of cranberry pomace extruded at different percentages of pomace. Values with different letters are significantly different (p < 0.05). An asterisk indicates values that are significantly different (p < 0.05) from an unextruded control.

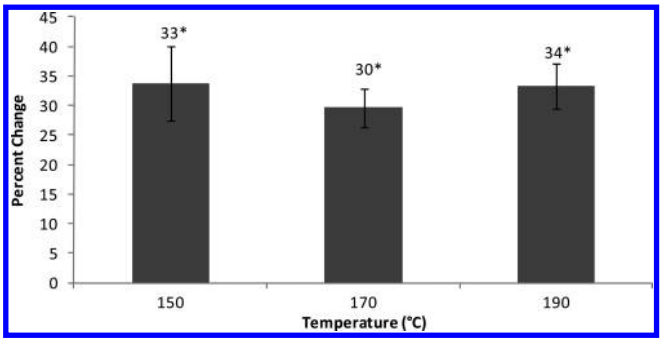

Figure 3. Change in total flavonol content of cranberry pomace extruded at different temperatures. An asterisk indicates values that are significantly different (p < 0.05) from an unextruded control.

30% pomace, with only 50% of anthocyanins lost. Extrudates containing 50% pomace lost the greatest amount of anthocyanins, with only 35% retention. This suggests possible protection of anthocyanins by the starch present in the extrudate mixture.

Flavonols. The flavonol composition of the cranberry pomace is presented in **Table 1**. Thirteen flavonols were identified in the pomace including glycosides of myricetin and quercetin as well as the aglycones, which were present in high amounts. **Figure 3** shows the change in total flavonol content for each barrel temperature. An increase in total flavonols was observed upon extrusion at all conditions when compared to an unextruded control, but there were no significant differences among the conditions and no interactions among the factors. The extrudates contained 30−34% more total flavonols than the control.

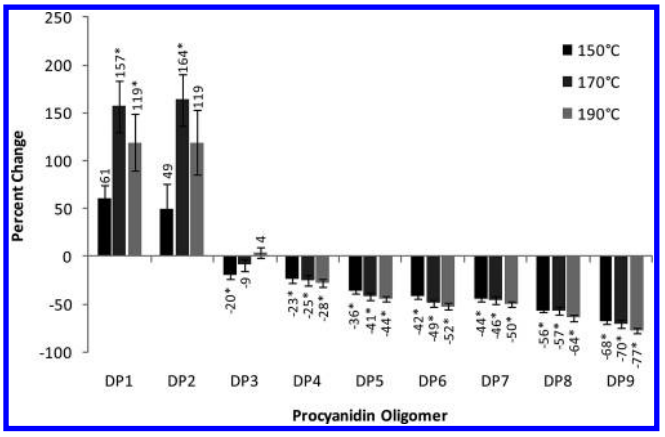

Figure 4. Change in procyanidin oligomer content of cranberry pomace extruded at different temperatures. An asterisk indicates values that are significantly different ($p < 0.05$) from an unextruded control. DP1 = monomer, DP2 = dimer, DP3 = trimer, etc.

The greatest increase was observed in quercetin 3-rhamnoside; however, all others increased as well. Although there has been limited research regarding the stability of flavonols, this suggests that flavonols are heat stable compared to the anthocyanins. The heat stability of quercetin in onions was demonstrated by baking and sautéing as researchers observed 7−25% increases in the quercetin content (*24*). Researchers attributed the increases to a concentration effect upon cooking. However, in this study, moisture losses were accounted for. Therefore, the increase in total flavonols may be explained otherwise. There is evidence that many flavonols may be bound to cell wall components, especially after injury to the cells (*25*). Therefore, the apparent increase in total flavonols that we observed in this study may be explained by enhanced extraction of the compounds due to disruption of the pomace matrix upon extrusion.

Procyanidins. Cranberry pomace contains procyanidins with DP1−DP9 (**Table 1**), with dimers predominating. Changes in procyanidin distribution due to extrusion were observed and varied according to the DP of the procyanidin. No significant differences were observed among the treatments; however, when treatments were compared to the control, differences did exist. Additionally, there were no significant interactions among the factors. Changes in content of each procyanidin oligomer at different temperatures are displayed in **Figure 4**. Increases in DP1 and DP2 procyanidins were observed as a result of extrusion. DP1 procyanidins increased 61−157%, whereas DP2 procyanidins increased 49−164%. A decrease in DP3 procyanidins was observed at 150 °C, but there were no significant changes under any of the other extrusion conditions. A significant reduction in procyanidins with DP ≥ 4 was observed in the extrudates for all conditions. Procyanidin losses were apparent as procyanidin DP increased, with 23−28% losses of DP4 procyanidins and 68−77% losses of DP9 procyanidins.

These results are similar to those observed in extruded blueberry pomace and white sorghum mixtures, in which extrusion increased the monomer, dimer, and trimer contents of the blueberry pomace (*26*). Similar results were also observed in extruded sorghum grain. Procyanidins with DP ≤ 4 increased upon extrusion, whereas above DP5, a reduction in procyanidins was observed (*27*). Loss and redistribution of isoflavone content were observed when wheat flour was extruded with with wet okara. Increases in genistin and daidzin were observed, which is believed to be the result of cleavage of the malonyl group from malonyl daidzin and malonyl genistin (*28*). Additionally, when dark buckwheat was extruded, the HPLC profile of phenolics was altered, therefore leading researchers to believe there was a change in the composition of the phenolic compounds (*29*). This suggests that extrusion cooking is capable of breaking covalent bonds in phenolic compounds.

Very little is known about the interaction of polyphenolics, particularly procyanidins, with other plant components. The ability of procyanidins to bind proteins is what gives many plant foods their astringency. As a fruit matures, the level of extractable procyanidin oligomers decreases significantly (*30*, *31*). These compounds are either further metabolized or bound so strongly to other components of the plant, such as the cell wall, that they are no longer easily extracted. The latter theory is supported by research performed on procyanidins and apple cell wall material, where procyanidins were shown to readily bind to a suspension of cell wall material, with degree of binding increasing as the degree of polymerization of the procyanidin oligomer increased (*32*). A method has been developed to determine the amount of unextractable procyanidins in plant materials (*33*). The proportion of unextractable procyanidins of total procyanidin content determined using this method varied among plant materials with a table grape containing 63% and a cultivar of apple containing 4.1% unextractable procyanidins. On the basis of this theory of the presence of unextractable procyanidins, it is possible that the increases we observed in low molecular weight procyanidins are due to alteration of the plant cell wall material present in the pomace, which, in turn, facilitated an increase in extraction of the compounds in extruded material. However, we cannot rule out the possibility that large molecular weight procyanidins were depolymerized to monomers and dimers in response to the heat and shear incurred during extrusion.

The increase in low molecular weight procyanidins observed in this study is important because procyanidin absorption is largely dependent on the size of the molecule. Procyanidins larger than trimers have been shown to be poorly absorbed due to their size (*12*). Holt and others (*34*) detected catechin and epicatechin as well as a procyanidin dimer in the plasma of human subjects as early as 30 min after they consumed a cocoa beverage. One study determined that cacao procyanidins decreased diabetes-induced cataracts and found that levels of epicatechin and its metabolites reached their highest levels in the plasma between 1 and 2 h, but the B2 dimer was poorly absorbed (*35*). However, even if large molecular weight molecules are not absorbed, they may still protect against certain diseases of the intestine, such as cancer, by inhibiting oxidation in an area that is not typically rich in dietary antioxidants (*5*). Additionally, these compounds may be converted to smaller molecules such as phenolic acids by colonic microflora, which could subsequently be absorbed to provide additional health benefits (*36*).

Antioxidant Capacity. The antioxidant capacities of the extrudates and an unextruded control were determined using the ORAC assay. Cranberries are known to have a high antioxidant capacity due to the presence of flavonoids (anthocyanins, flavonols, and flavanols) and phenolic acids. Extracts from cranberries have been reported to inhibit LDL oxidation (*37*) and oxidative and inflammatory damage to the vascular endothelium (*38*). ORAC values for whole cranberry and a cranberry extract were determined to be 275 and 106 μmol of TE/g of dry matter, respectively (*6*). The ORAC value for unextruded cranberry pomace was determined to be 281.3 ± 25.8 μmol of TE/g of dry matter.

ORAC values were found to be dependent upon the barrel temperature of the extruder (**Figure 5**). ORAC values increased with increasing temperature, and extrudates produced at 170 and 190 °C had ORAC values 16 and 30% higher than the control values, respectively. Similarly, the antioxidant activity of dark buckwheat extrudates was not significantly different from the raw

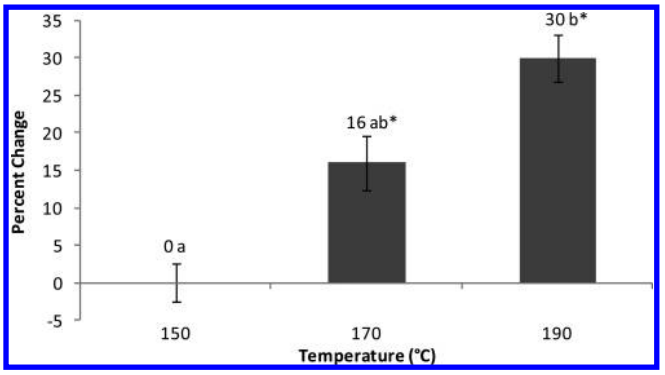

Figure 5. Change in antioxidant capacity of cranberry pomace extruded at different temperatures. Values with different letters are significantly different ($p < 0.05$). An asterisk indicates values that are significantly different ($p < 0.05$) from an unextruded control.

dark buckwheat (*29*). However, the antioxidant capacity of an extruded snack food was negatively affected (*39*). The higher ORAC values of extrudates obtained at elevated barrel temperatures of 170 and 190 °C were most likely due to the formation of Maillard reaction products, which possess reducing capacity (*40*). We believe this is the case because polyphenolics did not correlate well with ORAC. The extrudates obtained at 190 °C were visibly darker than the other samples, indicating Maillard browning.

Extrusion of cranberry pomace resulted in significant losses of total anthocyanins. However, increases in flavonols and low DP (1−2) procyanidins and a decrease in high DP (4−9) procyanidins were observed. The antioxidant capacity of the extrudates increased at higher temperatures. Applications of this research could provide a use for the waste product of cranberry juicing, which currently has little functionality due to its low protein content and low pH. Furthermore, it could lead to the improved functionality of polyphenolic compounds, particularly procyanidins and flavonols, of cranberry pomace. However, means of curbing the loss of anthocyanins due to heat need to be evaluated. The resulting product could be incorporated into a dietary supplement or explored as a functional snack food.

ACKNOWLEDGMENT

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NOTE ADDED AFTER ASAP PUBLICATION

Changes were made to the HPLC Analysis of Flavonols paragraph after the original Web publication of December 18, 2009. These changes are reflected in the posting of December 28, 2009.

LITERATURE CITED

(1) Vattem, D. A.; Shetty, K. Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. *Food Biotechnol*. **2002**, *16*, 189–210.

(2) Howell, A. B.; Leahy, M.; Kurowska, E.; Guthrie, N. In vivo evidence that cranberry proanthocyanidins inhibit adherence of p-fimbriated *E. coli* bacteria to uroepithelial cells. *Fed. Am. Soc. Exp. Biol. J*. **2001**, *15*, A284.

(3) Seeram, N. P.; Adams, L. S.; Hardy, M. L.; Heber, D. Total cranberry extract vs its phytochemical consituents: antiproliferative and synergistic effects against human tumor cell lines. *J. Agric. Food Chem*. **2004**, *52*, 2512–2517.

(4) Chu, Y.; Liu, R. H. Cranberries inhibit LDL oxidation and induce LDL receptor expression in hepatocytes. *Life Sci*. **2005**, *77*, 1892–1901.

(5) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr*. **2004**, *79*, 727–747.

(6) Prior, R. L.; Lazarus, S. A.; Cao, G.; Muccitelli, H.; Hammerstone, J. F. Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem*. **2001**, *49*, 1270–1276.

(7) Drewnowski, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr*. **2000**, *72*, 1424–1435.

(8) Vvedenskaya, I. O.; Rosen, R. T.; Guido, J. E.; Russell, D. J.; Mills, K. A.; Vorsa, N. Characterization of flavonols in cranberry (*Vaccinium macrocarpon*) powder. *J. Agric. Food Chem*. **2004**, *52*, 188–195.

(9) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem*. **2003**, *51*, 7513–7521.

(10) Howell, A. B.; Vorsa, N.; Marderosian, A. D.; Foo, L. Y. Inhibition of the adherence of p-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. *N. Engl. J. Med*. **1998**, *339*, 1085–1086.

(11) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem*. **2002**, *50*, 4852–4860.

(12) Donovan, J. L.; Manach, C.; Rios, L.; Morand, C.; Scalbert, A.; Remesy, C. Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. *Br. J. Nutr*. **2002**, *87*, 299–306.

(13) Gonthier, M. P.; Donovan, J. L.; Texier, O.; Felgines, C.; Remesy, C.; Scalbert, A. Metabolism of dietary procyanidins in rats. *Free Radical Biol. Med*. **2003**, *35*, 837–844.

(14) Garcia-Ramirez, B.; Fernandez-Larrea, J.; Salvado, M. J.; Ardevol, A.; Arola, L.; Blade, C. Tetramethylated dimeric procyanidins are detected in rat plasma and liver early after oral administration of synthetic oligomeric procyanidins. *J. Agric. Food Chem*. **2006**, *54*, 2543–2551.

(15) Shoji, T.; Masumoto, S.; Moriichi, N.; Akiyama, H.; Kanda, T.; Ohtake, Y.; Goda, Y. Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the Porter method and high-performance liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem*. **2006**, *54*, 884–892.

(16) Berrios, J. D. J.; Camara, M.; Torija, M. E.; Alonso, M. Effect of extrusion cooking and sodium bicarbonate addition on the carbohydrate composition of black bean flours. *J. Food Process. Preserv*. **2002**, *26*, 113–128.

(17) Huber, G. Twin-screw extruders. In *Extruders in Food Applications*; Riaz, M. N., Ed.; CRC Press: Boca Raton, FL, 2000; pp 81−114.

(18) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *Am. Soc. Nutr. Sci*. **2003**, 613–617.

(19) Kelm, M. A.; Johnson, J. C.; Robbins, R. J.; Hammerstone, J. F.; Schmitz, H. H. High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *J. Agric. Food Chem*. **2006**, *54*, 1571–1576.

(20) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric*. **2004**, *84*, 1771–1782.

(21) Prior, R. L.; Xianli, W.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem*. **2005**, *53*, 4290–4302.

(22) Camire, M. E.; Dougherty, M. P.; Briggs, J. L. Functionality of fruit powders in extruded corn breakfast cereals. *Food Chem*. **2007**, *101*, 765–770.

(23) Chaovanalikit, A.; Dougherty, M. P.; Camire, M. E.; Briggs, J. Ascorbic acid fortification reduces anthocyanins in extruded blueberry−corn cereals. *J. Food Sci.* **2003**, *68*, 2136–2140.

(24) Lombard, K.; Peffley, E.; Geoffriau, E.; Thompson, L.; Herring, A. Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. *J. Food Compos. Anal.* **2005**, *18*, 571–581.

(25) Hutzler, P.; Fischbach, R.; Heller, W.; Jungblut, T.; Reuber, S.; Schmitz, R.; Veit, M.; Weissenbock, G.; Schnitzler, J. Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. *J. Exp. Bot.* **1998**, *49*, 953–965.

(26) Khanal, R. C.; Howard, L. R.; Brownmiller, C. R.; Prior, R. L. Influence of extrusion processing on procyanidin composition and total anthocyanin contents of blueberry pomace. *J. Food Sci.* **2009**, *74*, H52–H58.

(27) Awika, J. M.; Dykes, L.; Gu, L.; Rooney, L. W.; Prior, R. L. Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. *J. Agric. Food Chem.* **2003**, *51*, 5516–5521.

(28) Rinaldi, V. E. A.; Ng, P. K. W.; Bennink, M. R. Effects of extrusion on dietary fiber and isoflavone contents of wheat extrudates enriched with wet okara. *Cereal Chem.* **2000**, *77*, 237–240.

(29) Senoy, I.; Rosen, R. T.; Ho, C.; Karwe, M. V. Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chem.* **2006**, *99*, 388–393.

(30) Czochanska, Z.; Foo, L. Y.; Porter, L. J. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **1979**, *18*, 1819–1822.

(31) De Freitas, V. A. P.; Glories, Y. Concentration and compositional changes of procyanidins in grape seeds and skin of white *Vitis vinifera* varieties. *J. Sci. Food Agric.* **1999**, *79*, 1601–1606.

(32) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material: Part I. Effect of some environmental parameters. *Biochim. Biophys. Acta* **2004**, *1672*, 192–202.

(33) Hellström, J. K.; Mattila, P. H. HPLC determination of extractable and unextractable proanthocyanidins in plant materials. *J. Agric. Food Chem.* **2008**, *56*, 7617–7624.

(34) Holt, R. R.; Lazarus, S. A.; Sullards, M. C.; Zhu, Q. Y.; Schramm, D. D.; Hammerstone, J. F.; Fraga, C. G.; Schmitz, H. H.; Keen, C. L. Procyanidin dimer B2 [epicatechin-(4β-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am. J. Clin. Nutr.* **2002**, *76*, 798–804.

(35) Osakabe, N.; Yamagishi, M.; Natsume, M.; Yasuda, A.; Osawa, T. Ingestion of proanthocyanidins derived from cacao inhibits diabetes-induced cataract formation in rats. *Exp. Biol. Med.* **2004**, *229*, 33–39.

(36) Deprez, S.; Brezillion, C.; Rabot, S.; Philippe, C.; Mila, R.; Lapierre, C.; Scalbert, A. Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* **2000**, *130*, 2733–2738.

(37) Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **2002**, *50*, 5844–5849.

(38) Youdim, K. A.; McDonald, J.; Kalt, W.; Joseph, J. A. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J. Nutr. Biochem.* **2002**, *13*, 282–288.

(39) Ozer, E. A.; Herken, E. N.; Guzel, S.; Ainsworth, P.; Ibanoglu, S. Effect of extrusion process on the antioxidant activity and total phenolics in a nutritious snack food. *Int. J. Food Sci.* **2006**, *41*, 289–293.

(40) Yilmaz, Y.; Toledo, R. Antioxidant activity of water-soluble Maillard reaction products. *Food Chem.* **2005**, *93*, 273–278.

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