JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Release and Degradation of Anthocyanins and Phenolics from Blueberry Pomace during Thermal Acid Hydrolysis and Dry Heating

Mustafa Bener,[†] Yixiao Shen,[§] Reşat Apak,[†] John W. Finley,[§] and Zhimin Xu*[,§]

[†]Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar 34320, Istanbul, Turkey

[§]Department of Food Science, Agricultural Center, Louisiana State University, Baton Rouge, Louisiana 70803, United States

ABSTRACT: In this study, blueberry pomace was soaked in pH 1, 4, or 7 solution for 10 min followed by boiling hydrolysis. Nine anthocyanins and 11 other phenolic compounds were released after acid hydrolysis. The highest anthocyanin release (4.70 mg/g) was achieved by boiling at pH 1 for 15 min followed by 3.94 mg/g at pH 4 and 3.46 mg/g at pH 7. Phenolics were released more quickly than anthocyanins during boiling. The change of antioxidant activity of the pomace during boiling was correlated with the total phenolic content but not anthocyanin content. The degradation rate of anthocyanins during boiling eventually surpassed the release rate from the pomace. Protocatechuic acid and catechin continuously increased during heating. Dry heat resulted in continuous degradation of anthocyanins and other phenolics in the pomace. The reduction in antioxidant activity of the pomace during dry heating was correlated with both the phenolic and anthocyanin contents.

KEYWORDS: *pomace, anthocyanins, antioxidants, phenolics, blueberry*

INTRODUCTION

Blueberries are rich sources of anthocyanins and other phenolics of antioxidant compounds, which may have health-promoting functionality.[1] As blueberries have a limited shelf life, a large portion of fresh blueberries are processed into juice. The juice processing generates a large quantity of pomace. The pomace consists of skins, pulp residue, and seeds. It is usually treated as a waste product, some of which goes to animal feed, but more is used as field dressing or is placed in landfills. However, the pomace has potential value because it retains a significant amount of bound anthocyanins and phenolics.[2] The pomace can be processed into an interesting new food ingredient by simply grinding it to reduce the coarse texture. Bound anthocyanins within the fiber may not be readily utilized by the body because of their lower bioaccessibility in the gastrointestinal tract. A rational approach to more effectively utilize the health-promoting anthocyanins and other phenolics in the fruit pomace is to establish a simple means to release them from the fiber with minimal degradation. The free anthocyanin-rich pomace could then be used as a functional food ingredient or nutritional supplement with enhanced delivery of the anthocyanins and fiber from the pomace.

Acid hydrolysis is frequently used to release bound phytochemicals from a fiber matrix. Boiling of the matrix has also been applied to enhance the efficiency of acid hydrolysis. The stability of anthocyanins was reported to be variable at different pH levels and temperatures.[3−5] Variability in the type of the matrix fiber may also influence the release and stability of the bound anthocyanins and other phenolics. In this study, we investigated and optimized an acid hydrolysis and boiling process to obtain the greatest release of anthocyanins from blueberry pomace. The release of anthocyanins from blueberry pomace was investigated at pH 1, 4, and 7 and compared. Changes in total phenolic and antioxidant activity in the hydrolyzed pomace were also monitored. The anthocyanin-rich pomace is likely used as an ingredient in breakfast cereals or bakery products, which are usually extruded at a high temperature.[6−8] Changes in anthocyanins, other phenolics, and antioxidant activity in the hydrolyzed pomace during dry heating at different high temperatures were also investigated to simulate cereal processing conditions. The goal of this study is to produce an anthocyanin-rich blueberry pomace that is more thermally stable yet delivers available anthocyanins.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is a common method for evaluating the antioxidant activity of phytochemicals or plant extracts.[9] DPPH is a free radical generator and has absorbance at 515 nm. The decreased rate of DPPH absorbance at 515 nm is correlated to the antioxidant activity or free radical scavenging capability of the tested sample. However, the absorbance at 515 nm is prone to interference by other components, such as anthocyanins, in the test sample which has absorption in that wavelength range. The DPPH method is not an ideal assay to determine the antioxidant activity of anthocyanin-rich pomace samples. In this study, a sensor-based cupric reducing antioxidant capacity (CUPRAC) method was applied to measure the antioxidant activity of blueberry pomace during boiling and dry heating experiments. The principle of this method relies on the redox reaction of Cu(II)−neocuproin to Cu(I)−neocuproin. The absorbance of the generated colored chelate of Cu(I)−neocuproin at 450 nm is used for calculating antioxidant activity.[10] The method is not influenced by pH or color components in the test sample.[11] Thus, the CUPRAC method is more suitable than DPPH for evaluating the antioxidant activity of the anthocyanin rich samples.

Received: May 6, 2013
Revised: June 11, 2013
Accepted: June 17, 2013
Published: June 17, 2013

MATERIALS AND METHODS

Chemicals. Copper(II) chloride dihydrate, copper(II) sulfate, neocuproine (2,9-dimethyl-1,10-phenanthroline), potassium sodium tartrate tetrahydrate, Folin−Ciocalteu reagent, trolox, cyanidin chloride, and phenolic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate and glacial acetic acid were purchased from Fisher Scientific (Springfield, NJ, USA). Sodium carbonate was purchased from J. T. Baker Chemical (Phillipsburg, NJ, USA). Sodium hydroxide was purchased from EM Science (Gibbstown, NJ, USA). Hydrochloric acid, methanol, acetonitrile, and ethanol were from EMD Millipore (Billerica, MA, USA). Nafion 115 perfluorinated membrane (thickness = 0.005 in.) was purchased from Aldrich (Steinheim, Germany).

Blueberry Pomace Preparation. Fresh blueberries were obtained from a local grocery store. Two kilograms of blueberry was ground in a blender (Waring, Stamford, CT, USA) and transferred to a bucket. The blended blueberry slurry was filtered using cheesecloth to obtain the blueberry pomace. Then, the pomace was mixed with 5 kg of distilled water in the bucket to rinse the pomace. After the mixture was agitated for 10 min, the water was decanted. The rinsing step was repeated until the wash water became clean and colorless. Then, the rinsed anthocyanin pomace was spread on aluminum foil and dried under a hood at room temperature (22−25 °C). The dried pomace (moisture content = 11.7%) was stored at −4 °C until use.

Soaking and Boiling Hydrolysis. Acid solutions of pH 1 and 4 were prepared using hydrochloric acid and distilled water. Twenty-five grams of the dried pomace was mixed with 100 mL of the acid solution or neutral distilled water in a 250 mL flask. The mixture was agitated by a magnetic stir bar for 10 min. The flasks were then connected to a reflux cold condenser and placed in a boiling water bath. One milliliter of the solution was taken from the sampling hole of the flask at 0, 15, 30, and 45 min of boiling hydrolysis, respectively. The anthocyanin and phenolic contents and their antioxidant activities in the collected samples were determined according to the methods described below. All measured results from the samples were multiplied by the total hydrolysis solution volume (100 mL) and then divided by the amount of the pomace (25 g) to convert the results on the basis of dried pomace weight. Each treatment or control was repeated in triplicate.

Dry Heating Treatment for the Hydrolyzed Pomace. The sample with the highest free anthocyanin content in the previous hydrolysis study was used for the dry heating experiments. The solution with the hydrolyzed pomace was immediately cooled, adjusted to pH 4, poured into a cell culture dish (24.5 cm × 24.5 cm), and dried at 60 °C in a convection oven. The dried blueberry pomace was ground to fine powder. Then, 0.2 g of the powder was added to each of nine test tubes for dry heating treatments. The test tubes were inserted in a sand bath, which was set at 175, 200, or 225 °C (High-Temp Bath 160 A, Fisher Scientific). Three of the nine test tubes were taken from the sand bath at 5, 10, and 15 min. The anthocyanin and total phenolic contents and their antioxidant activities in the heated and unheated pomace samples were determined.

Determination of Anthocyanins and Other Phenolics. Anthocyanins and other phenolics were analyzed using the HPLC method described in the study of Yue and Xu.[8] The HPLC system consisted of a Waters 2690 separation module, a 996 photodiode array detector, and a Millenium32 chromatography manager (2690, Waters, Torrance, CA, USA) as well as a Luna C18 column (i.d. 250 × 4.60 mm, 5 μm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile (A) and 10% acetic acid (B) and was run in a linear gradient mode of ramping A from 0 to 50% in 50 min, decreasing A from 50 to 0% in 1 min, and holding A at 0% for 4 min. The detector was set at 520 nm for monitoring anthocyanins. The wavelengths for monitoring other phenolics are listed in Table 2. Each anthocyanin was identified by comparison of elution order with the studies of Yue and Xu[8] and Castaneda-Ovando et al.[7] The concentration of each anthocyanin was calculated by the calibration curve of cyanidin chloride in molar concentration and converted to micrograms per gram of pomace based on its molecular weight. The concentrations of other phenolics were calculated by the external calibration curves of their corresponding standards.

Determination of Total Phenolic Content (TPC). TPC was determined using the Folin−Ciocalteu method.[12] The solutions used in the assay were prepared as follows: (A) 2% aqueous Na_2CO_3 in 0.1 M NaOH; (B) 0.5% $CuSO_4$ aqueous solution in 1% potassium sodium tartrate tetrahydrate ($NaKC_4H_4O_6$) solution; (C) a mixture of 50 mL of A and 1 mL of B. The Folin−Ciocalteu reagent was diluted with H_2O at a volume ratio of 1:3 prior to use. The sample (0.1 mL) was mixed with 0.9 mL of distilled water and 2.5 mL of C solution in a test tube. Then, 0.25 mL of Folin−Ciocalteu reagent was added and vortexed. After 30 min, the absorbance of the reaction solution was measured at 750 nm. The result was converted to Trolox equivalent (mmol TR/g sample) units based on the standard curve of Trolox.

Determination of Antioxidant Activity (AC). The sensor-based CUPRAC method was used in determining the AC.[11] The reagents of the optical sensor-based CUPRAC method were prepared as follows: $CuCl_2$ solution (10 mM); ammonium acetate buffer at pH 7.0 [1.0 M neocuproine fresh (Nc) solution (0.75 mM in ethanol)]. Nafion is a perfluorosulfonate ion exchange membrane with R−{−O−CF_2−$CF(CF_3)$−}$_x$−O−$(CF_2)_2SO_3H$ functional groups. Nafion membrane was cut into 4.5 × 0.5 cm pieces and dipped into a tube that contained 2 mL of each solution above. Then, 2.2 mL of distilled water was added to the tube. After the tube was shaken for 30 min, the reagent saturated membrane (Nafion−Cu(II)−Nc) was taken out and immersed in a tube containing 0.12 mL of sample solution and 8.08 mL of ethanol. The tube was agitated for 30 min to carry out the dyeing reaction of the membrane. The absorbance of the colored membrane (Nafion−Cu(I)−Nc) was measured at 450 nm. A membrane was prepared under identical conditions without the sample as a blank. The antioxidant activity was expressed by using Trolox equivalent (mmol TR/g sample) units based on the standard curve of Trolox.

Data Analysis. Each treatment or control experiment was carried out in triplicates. The means, standard deviations, and linear regression coefficients were calculated using Excel (Microsoft Inc., Redmond, WA, USA). The data were analyzed by one-way ANOVA (SAS, 9.1.3, Cary, NC, USA) to evaluate the significant difference (at $P < 0.05$) between the results from the treatment and control groups.

RESULTS AND DISCUSSION

Release and Degradation of Bound Anthocyanins from Blueberry Pomace during Soaking and Boiling Hydrolysis. Nine anthocyanins (delphinidin galactoside, glucoside, and arabinoside; cyanidin galactoside and arabinoside; petunidin galactoside and glucoside; malvidin galactoside; and peonidin glucoside) were released from the completely rinsed pomace after acid hydrolysis (Figure 1 and Table 1). Petunidin glucoside and peonidin glucoside were the two major anthocyanins released, constituting approximately 60% of the total anthocyanins. The anthocyanins released in solution increased from undetectable level at the beginning to 1.65 mg/g at pH 7, 2.13 mg/g at pH 4, and 4.36 mg/g at pH 1 after 10 min of soaking (Figure 2). Unlike earlier studies of pH lability of anthocyanins, the measurements in this study were individual anthocyanins using HPLC rather than total anthocyanins by spectrophotometric methods. The absorbance of anthocyanins was found to be very sensitive to pH.[7,13] Thus, the same anthocyanin distribution in different pH solutions could be prone to variations in results due to the pH influence on the absorbance read from spectrophotometer. In this study, the higher acidity of the HPLC mobile phase could eliminate the influence of pH on sample solution. The HPLC method maintained a consistent low-pH condition for quantifying anthocyanins regardless of the pH of the original solution. Compared with the traditional spectrophotometric method, the HPLC method provided more reliable and accurate results in

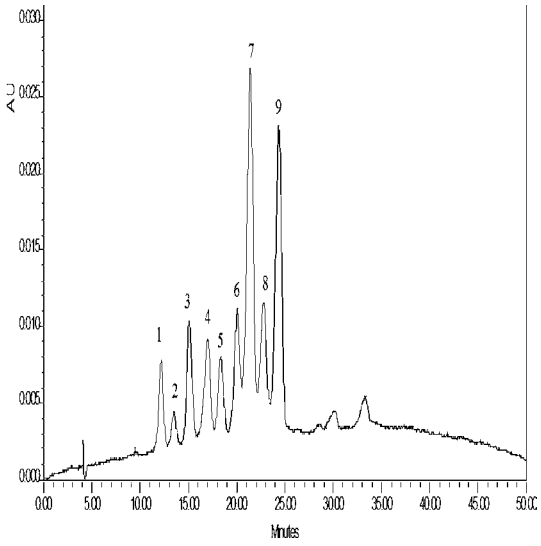

Figure 1. HPLC chromatogram of the anthocyanins released from blueberry pomace after soaking at pH 1 solution for 10 min. Peaks: 1, delphinidin galactoside; 2, delphinidin glucoside; 3, cyanidin galactoside; 4, delphinidin arabinoside; 5, petunidin galactoside; 6, cyanidin arabinoside; 7, petunidin glucoside; 8, malvidin galactoside; 9, peonidin glucoside.

Table 1. Anthocyanins Released from Pomace after Acid Hydrolysis

anthocyanin name	concentration (μg/g)
delphinidin galactoside	173.9 ± 2.7
delphinidin glucoside	70.3 ± 2.7
cyanidin galactoside	314.4 ± 77.3
delphinidin arabinoside	74.1 ± 16.9
petunidin galactoside	211.1 ± 11.2
cyanidin arabinoside	442.0 ± 13.8
petunidin glucoside	1408.9 ± 88.3
malvidin galactoside	473.7 ± 27.6
peonidin glucoside	1192.2 ± 75.5
total	4360.6 ± 316.0

this type of anthocyanin study. As shown in Figure 2, lower pH hydrolysis was more effective for releasing bound anthocyanins than higher pH values. The anthocyanin content reached the highest level (4.70 mg/g of pomace) after 15 min of boiling at pH 1, whereas the highest levels of the anthocyanin content were 3.94 and 3.46 mg/g for pH 4 and control, respectively, after 30 min of boiling (Figure 2). This suggested that acid hydrolysis with boiling could significantly increase the release of bound anthocyanins from the pomace. Extraction of anthocyanins from red radish with water and acidified water was studied by Patil et al.[14] Three solutions for the anthocyanin extraction, H_2O and 1% and 2% HCl, were used in their study. The results were similar to our study in that the higher acidity extraction solution had higher extraction yield of free anthocyanins.

The anthocyanin content was eventually found to decrease with increased boiling time (Figure 2). The anthocyanin contents in pH 1, 4, and 7 (control) decreased to similar levels at the end of 45 min of boiling. The results indicated that it was a dynamic process between the release and degradation of anthocyanins from blueberry pomace during boiling hydrolysis. The degradation rate ultimately surpassed the release rate as the

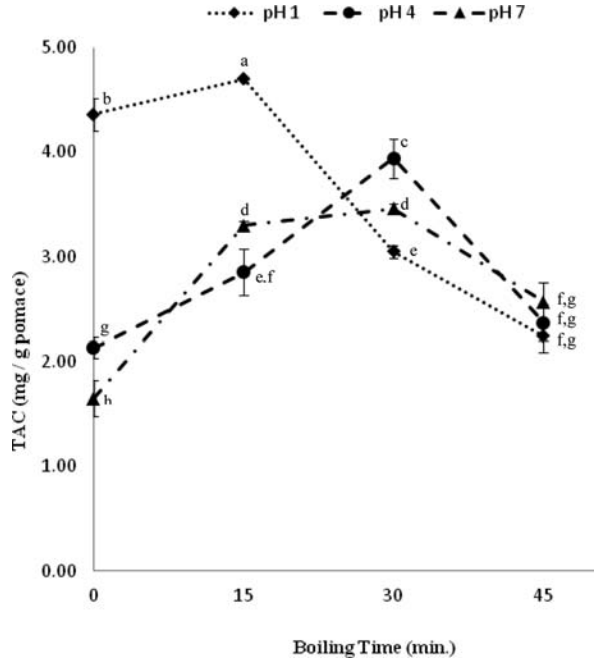

Figure 2. Total anthocyanin contents of blueberry pomaces during boiling hydrolysis at different pH treatment and control solutions. Data with different letters are significantly different in their values at $P < 0.05$.

boiling time was extended. The thermal stability of the anthocyanins of red-flesh sweet potato and grape pomace in different pH solutions was studied as well.[4,15] It was found that the anthocyanins of sweet potato at pH 3 solution were more stable than at pH 1 solution after 2 h at 98 °C. It was also reported that 60 and 64% of the anthocyanins in lotus root and onion were degraded after 60 min of boiling treatment.[16] These results were in accordance with our findings that boiling hydrolysis at stronger acid solution could also accelerate degradation of anthocyanins, although it was very effective in increasing the release of bound anthocyanins from the pomace.

Changes of the Anthocyanins and Other Phenolics from Blueberry Pomace during Boiling Hydrolysis. During acid hydrolysis, in addition to the release of anthocyanins, other phenolics including gallic acid, ellagic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, quercetin, and myricetin were liberated from the pomace (Table 2). Gallic acid, syringic acid, and quercetin were the

Table 2. Phenolics Released from Pomace after Acid Hydrolysis and Wavelengths for Their Quantification

anthocyanin name	concentration (μg/g)	wavelength (nm)
gallic acid	72.2 ± 2.1	272
ellagic acid	5.2 ± 0.5	250
protocatechuic acid	7.5 ± 0.6	260
chlorogenic acid	12.3 ± 1.0	326
caffeic acid	35.3 ± 1.4	324
syringic acid	82.1 ± 2.4	276
p-hydroxybenzoic acid	30.0 ± 3.4	290
p-coumaric acid	4.3 ± 0.3	309
ferulic acid	36.1 ± 2.5	324
quercetin	52.3 ± 2.8	370
myricetin	34.4 ± 1.1	370

dx.doi.org/10.1021/jf401983c | J. Agric. Food Chem. 2013, 61, 6643−6649

major nonanthocyanin phenolics. Phenolic compounds released during boiling at pH 1, 4, and 7 for different boiling times are shown in Figure 3. The boiling time for achieving the highest

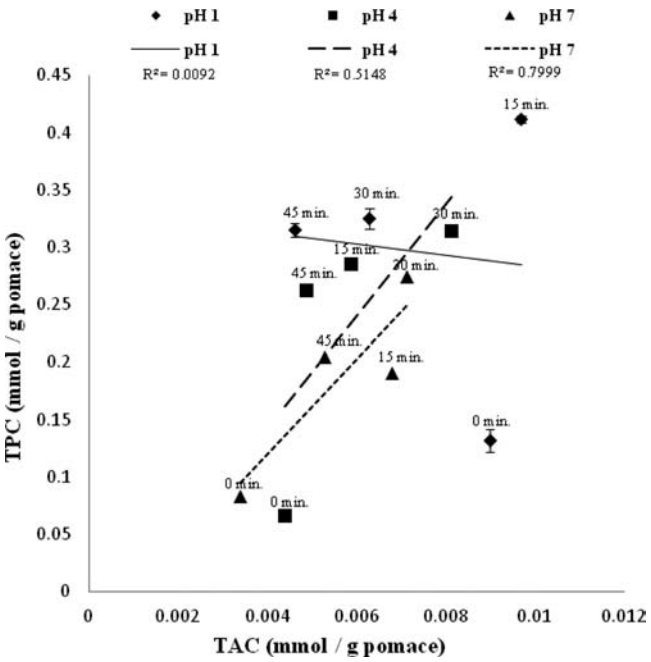

Figure 3. Correlations between total phenolics (TPC) and total anthocyanins contents (TAC) of blueberry pomace during boiling hydrolysis at different pH treatments and control solutions.

phenolic content in each treatment or control was identical to the time for obtaining the highest anthocyanin content. However, there was no correlation between phenolics and anthocyanins released during boiling hydrolysis. Their linear regression coefficients of the change of the phenolic content versus the change of the anthocyanin content were 0.0092, 0.5148, and 0.7999 in pH 1 and 4 and control groups, respectively (Figure 3). At pH 1, after the soaking hydrolysis, despite the presence of higher anthocyanin content of blueberry pomace, the phenolic content was not significantly higher than that at pH 4 or control solution. In other words, the effect of the pH difference in the soaking hydrolysis on the release of nonanthocyanin bound phenolics was not significant. The release of the phenolics was much faster than the release of anthocyanins from the pomace during the first 15 min of boiling. The boiling hydrolysis significantly accelerated the release of other bound phenolics rather than anthocyanins in the pomace. Like the anthocyanin content, the phenolic content decreased with boiling time increase. Compared with the decrease of the anthocyanin content, the decrease of the phenolic content was relatively slow. The reason may be that some anthocyanins could convert to other phenolic compounds during the boiling hydrolysis. In this study, two major degradation compounds, protocatechuic acid and catechin, were produced signficantly during the thermal treatment (Figure 4). The protocatechic acid and catechin contents increased to 79.0 and 30.0 μg/g pomace, respectviely, after the heating. Protocatechuic acid was also reported as a major degradation product of anthocyanins in the study of Rupasinghe et al.[17]

Changes of the Anthocyanin and Phenolic Contents of Hydrolyzed Blueberry Pomace during Dry Heating. Hydrolyzed blueberry pomace was prepared using the optimal hydrolysis condition (soaking with acidic solution pH 1 for 10 min fellowed by boiling for 15 min) obtained in the soaking and boiling study. During dry heating, the anthocyanin content was found to continuously decrease with heating time, and losses increased with increasing temperature (Figure 5). Compared with the initial content, the percentagesof retained anthocyanins after 15 min of dry heating were 42.29, 25.77, and 7.64% for 175, 200, and 225 °C, respectively. It was reported that the degradation constant of anthocyanins at 225 °C was several-fold higher than that at 175 °C.[8,18] The effect of dry heating at the relatively low temperature of 125 °C on the stability of anthocyanins in grape and blueberry pomace was reported by Khanal et al.[2] In their study, the anthocyanin content declined by 70 and 52% from their original levels in grape and blueberry pomace, respectively. The effect of baking temperature on cyanidin-3-galactoside-enriched muffin incor-

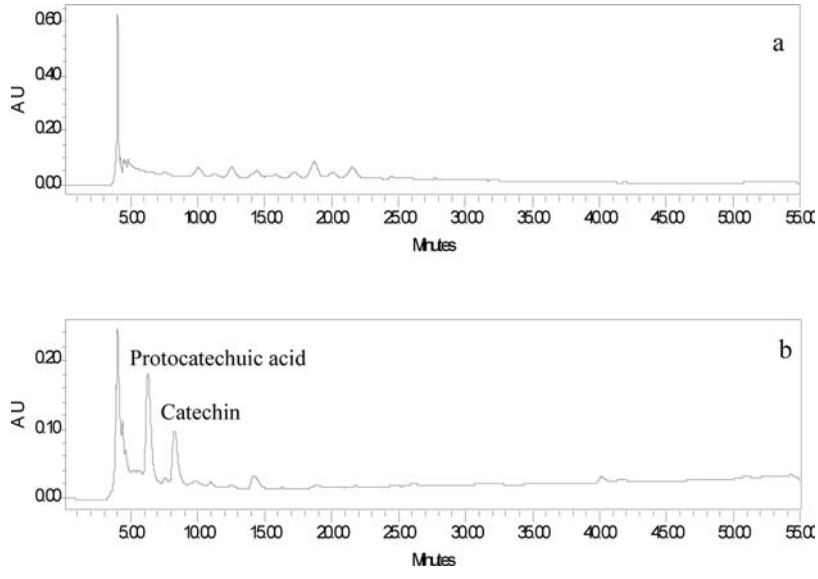

Figure 4. HPLC chromatograms of the phenolics in blueberry pomace before (a) and after (b) heating.

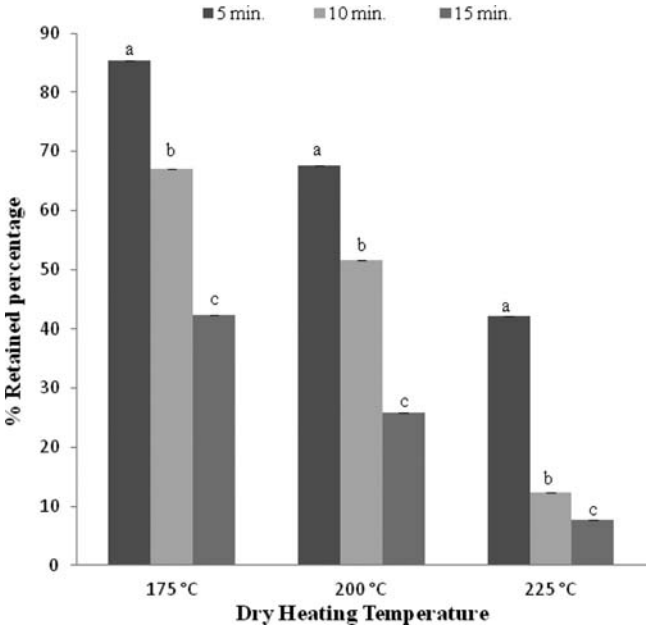

Figure 5. Percentages of the retained anthocyanins in the hydrolyzed blueberry pomaces during dry heating at different temperatures and times. Data at the same temperature with different letters are significantly different in their values ($P < 0.05$).

porated with apple skin powder was studied by Rupasinghe et al.[17] After 20 min of baking at 175 °C, the anthocyanin content only retained 15.7 and 24.8% for two different apple skin powder samples.

The phenolic content in the pomace also decreased with increasing dry heating time (Figure 6). However, unlike the boiling treatment, the decreased rates of the total phenolics and anthocyanins were correlated during the dry heating. The correlation coefficients of the phenolics and anthocyanins contents were 0.9695, 0.9956, and 0.9865 at 175, 200, and 225 °C, respectively (Figure 6). These results suggested that both the anthocyanins and other phenolics were seriously degraded and decreased at a similar rate at the severe dry heating temperatures. It may be possible that thermal transitions of anthocyanins to other small phenolics prior to complete degradation did not exist in the dry heating at high temperature. The finding was in agreement with the study of Yue and Xu that extreme dry heating temperature could cause total degradation of anthocyanins to small fragments.[8]

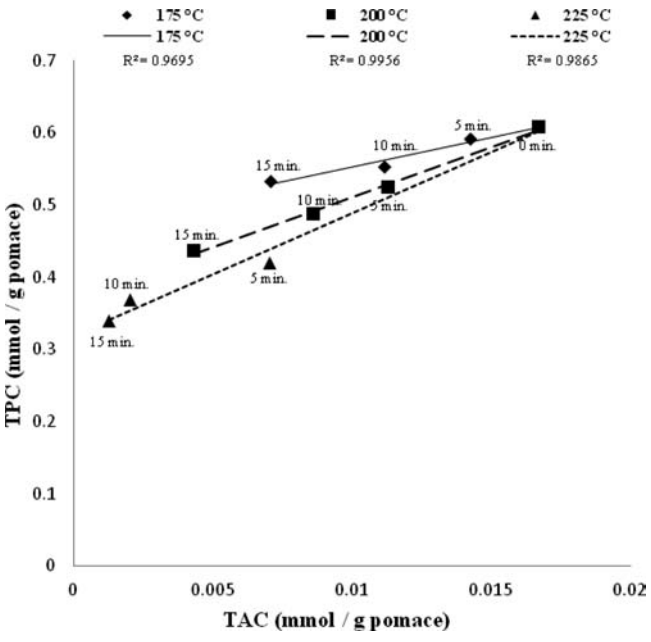

Figure 6. Correlations between the total phenolic (TPC) and anthocyanin contents (TAC) of the hydrolyzed blueberry pomace during dry heating at different temperatures and times.

Relationships of the Antioxidant Activity and Phenolic Content of Blueberry Pomace during Boiling Hydrolysis and Dry Heating. There was a strong correlation between antioxidant activity and phenolic content in the pomace during the boiling hydrolysis at different boiling times and pH levels (Figure 7). Correlation of the phenolic content and antioxidant activity of the colored vegetables and herbal teas was found by using the CUPRAC antioxidant activity assay in several previous studies.[19−21] These results indicated that the phenolics rather than the anthocyanins alone play an important role in contributing to the whole antioxidant activity. The reason may be that anthocyanins in pomace could transform into other types of phenolics which had higher levels of antioxidant activity during the boiling hydrolysis.[22,23] A strong correlation of antioxidant activity and phenolic contents of the hydrolyzed pomace was also found during dry heating at different temperatures (Figure 8). In the dry heating experiment, all phenolics including anthocyanins in the pomace were completely degraded to small fragments that had no antioxidant activity. Thus, the loss of the anthocyanins and other phenolics straightforwardly linked to the decrease of antioxidant activity of the dry-heated pomace.

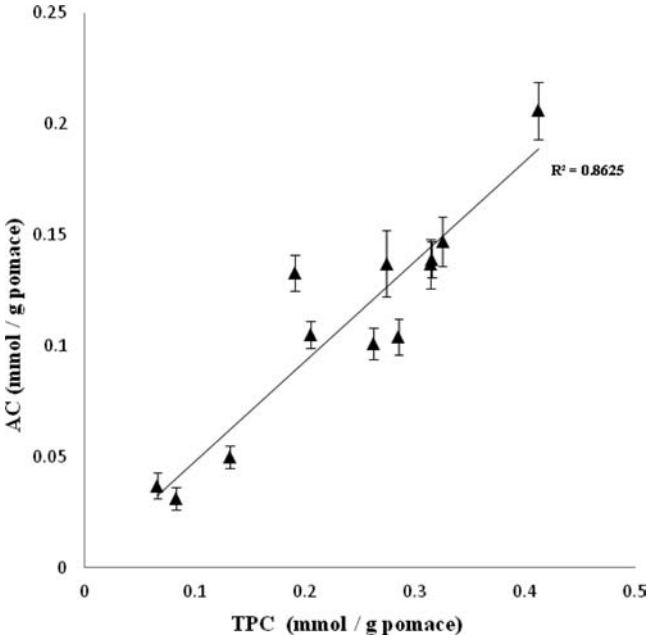

Figure 7. Correlation between the antioxidant activity (AC) and total phenolic content (TPC) of blueberry pomace during boiling hydrolysis at different pH treatments and control solutions.

In this study, the release and degradation of bound anthocyanins and other phenolics from blueberry pomace during soaking and boiling hydrolysis in different pH solutions and with dry heating at different temperatures (175, 200, or 225 °C) was studied. The release rate of anthocyanins and other

dx.doi.org/10.1021/jf401983c | J. Agric. Food Chem. 2013, 61, 6643−6649

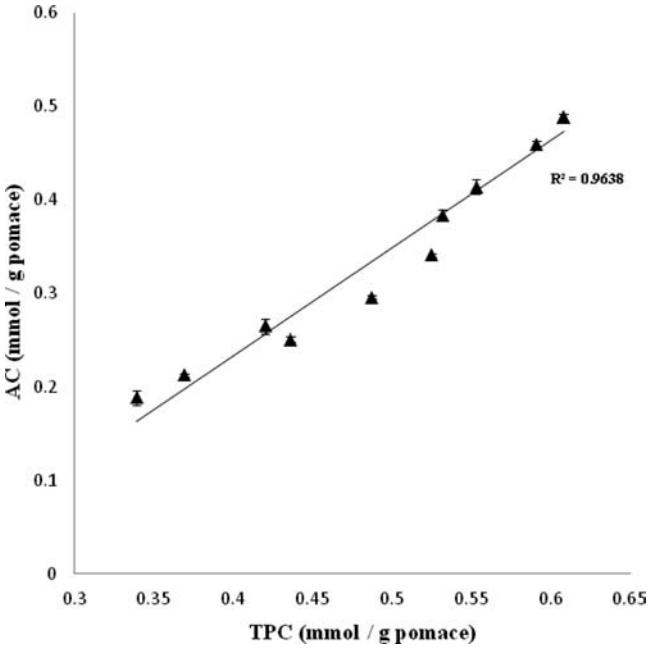

Figure 8. Correlation between antioxidant activity (AC) and total phenolic content (TPC) of the hydrolyzed blueberry pomace during dry heating at different temperatures and times.

phenolics increased with increasing acidity of the hydrolysis solution. Boiling treatment improved their release rate but also caused degradation of the released anthocyanins and other phenolics. The major degradation products of anthocyanins during boiling hydrolysis were protocatechuic acid and catechin. The optimal condition for obtaining the highest free anthocyanin content from blueberry pomace was soaking in a pH 1 solution for 10 min followed by 15 min of boiling. Compared with boiling hydrolysis, dry heating led to serious degradation of anthocyanins and other phenolics. The results of this study would be very useful for utilizing blueberry pomace as an anthocyanin- and fiber-rich food ingredient or nutrition supplement. They could also be guidance information for developing anthoycanin-rich blueberry pomace cereal or bakery food products with highly retained anthocyanins.

AUTHOR INFORMATION

Corresponding Author

*(Z.X.) Phone: (225) 388-5280. Fax: (225) 388-5300. E-mail: zxu@agcenter.lsu.edu.

Funding

M.B. thanks Istanbul University Research Fund, Bilimsel Arastirma Projeleri (BAP) Yurutucu Sekreterligi, for the support given to his Ph.D. thesis Project T-18392. M.B. also acknowledges TUBITAK (Turkish Scientific and Technical Research Council) for an International Postdoctoral Research Fellowship.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainlend, C. M. Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2868−2693.

(2) Khanal, R. C.; Howard, L. R.; Prior, R. L. Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins. *Food Res. Int.* **2010**, *43*, 1464−1469.

(3) Arozarena, I.; Ortiz, J.; Hermosin-Gutierrez, I.; Urretavizcaya, I.; Salvatierra, S.; Cordova, I.; Marin-Arroyo, M. R.; Noriega, M. J.; Navarro, M. Color, ellagitannins, anthocyanins, and antioxidant activity of Andean blackberry (*Rubus glaucus* Benth.) wines. *J. Agric. Food Chem.* **2012**, *60*, 7463−7473.

(4) Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem.* **2004**, *86*, 69−77.

(5) Howard, L. R.; Brownmiller, C.; Prior, R. L.; Mauromoustakos, A. Improved stability of chokeberry juice anthocyanins by β-cyclodextrin addition and refrigeration. *J. Agric. Food Chem.* **2013**, *61*, 693−699.

(6) Camire, M. E.; Chaovanalikit, A.; Dougherty, M. P.; Briggs, J. Blueberry and grape anthocyanins as breakfast cereal colorants. *J. Food Sci.* **2002**, *67*, 438−441.

(7) Castaneda-Ovando, A.; Pacheco-Hernandez, M. L.; Paez-Hernandez, M. A.; Rodriguez, J. A.; Galan-Vidal, C. A. Chemical studies of anthocyanins: review. *Food Chem.* **2009**, *113*, 859−871.

(8) Yue, X.; Xu, Z. Changes of anthocyanins, anthocyanidins, and antioxidant activity in bilberry extract during dry heating. *J. Food Sci.* **2008**, *73*, 494−499.

(9) Sanchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270−276.

(10) Apak, R.; Güçlü, K.; Özyürek, M.; Karademir, S. E. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.* **2004**, *52*, 7970−1981.

(11) Bener, M.; Özyürek, M.; Güçlü, K.; Apak, R. Development of a low-cost optical sensor for cupric reducing antioxidant capacity measurement of food extract. *Anal. Chem.* **2010**, *82*, 4252−4258.

(12) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(13) Malien-Aubert, C.; Dangles, O.; Amiot, M. J. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra and intermolecular copigmentation. *J. Agric. Food Chem.* **2001**, *49*, 170−176.

(14) Patil, G.; Madhusudhan, M. C.; Babu, B. R.; Raghavaro, K. S. M. S. Extraction, dealcoholization and concentration of anthocyanin from red radish. *Chem. Eng. Prog.* **2009**, *48*, 364−369.

(15) West, M. E.; Mauer, L. J. Color and chemical stability of a variety of anthocyanins and ascorbic acid in solution and powder forms. *J. Agric. Food Chem.* **2013**, *61*, 4169−4179.

(16) Im, M. H.; Park, Y. S.; Leontowicz, H.; Leontowicz, M.; Namiesnik, J.; Ham, K. S.; Kang, S. G.; Najman, K.; Gorinstein, S. The thermostability, bioactive compounds and antioxidant activity of some vegetables subjected to different durations of boiling: investigation in vitro. *LWT−Food Sci. Technol.* **2011**, *44*, 92−99.

(17) Rupasinghe, H. P. V.; Wang, L.; Huber, G. M.; Pitts, N. L. Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. *Food Chem.* **2008**, *107*, 1217−1224.

(18) Cisse, M.; Vaillant, F.; Acosta, O.; Dhuique-Mayer, C.; Dornier, M. Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using the Arrhenius, Erying, and Ball models. *J. Agric. Food Chem.* **2009**, *57*, 6285−6291.

(19) Apak, R.; Güçlü, K.; Demirata, B.; Özyürek, M.; Çelik, S. E.; Bektaşoğlu, B.; Berker, K. I.; Özyurt, D. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* **2007**, *12*, 1496−1547.

(20) Sun, T.; Xu, Z.; Wu, C. T.; Janes, M.; Prinyawiwatkul, W.; No, H. K. Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *J. Food Sci.* **2007**, *72*, S98−102.

(21) Apak, R.; Güçlü, G.; Özyürek, M.; Karademir, S. E.; Erçeğ, E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int. J. Food Sci. Nutr.* **2006**, *57*, 292−304.
(22) Song, B. J.; Sapper, T. N.; Burtch, C. E.; Brimmer, K.; Goldschmidt, M.; Ferruzzi, M. G. Photo- and thermodegradation of anthocyanins from grape and purple sweet potato model beverage system. *J. Agric. Food Chem.* **2013**, *61*, 1364−1372.
(23) Nayak, B.; Berrios, J. D. J.; Powers, J. R.; Tang, J. Thermal degradation of anthocyanins from purple potato (cv. Purple Majesty) and impact on antioxidant capacity. *J. Agric. Food Chem.* **2011**, *59*, 11040−11049.