DOI:10.1021/jf9021948

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Degradation of Anthocyanins and Anthocyanidins in Blueberry Jams/Stuffed Fish

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This study examined the effects of cooking on the degradation of anthocyanins and anthocyanidins of blueberries (*Vaccinium corymbosum* L.) from cultivar Bluecrop. Fruits were used to prepare jams with different °Brix and stuffed fish. A systematic evaluation of the degradation of anthocyanins and anthocyanidins of blueberries was performed; for that purpose an HPLC/DAD method was used to determine anthocyanin profile and anthocyanidin contents in fresh and cooked blueberries and in jams. Ten anthocyanins were separated and monitored in methanolic extracts. Of the six common anthocyanidins, four were identified in the hydrolysates, namely, delphinidin, cyanidin, petunidin and malvidin. Percentage of degradation of anthocyanins and anthocyanidins in jams is highly dependent on °Brix: 64−76 °Brix led to 20−30% degradation, whereas 80 °Brix resulted in degradation between 50 and 60%. Percentage of degradation of anthocyanins in whole blueberries cooked in stuffed fish ranged between 45 and 50%, however, for anthocyanidins, the percentage of degradation was significantly lower, between 12 and 30%, indicating that this cooking procedure can preserve anthocyanidin degradation.

KEYWORDS: Blueberry; anthocyanins; anthocyanidins; heat degradation; jam

1. INTRODUCTION

Berries, especially blueberries, have received much attention due to their possible health benefits as dietary antioxidant, antimutagenic, and chemopreventive nutraceuticals that contribute to reduced incidences of chronic diseases (*1*, *2*). These effects have generally been attributed to anthocyanins which are responsible for the blue, purple, violet, and red colors of fruit and are located in cells near the surface.

The anthocyanin pigments consist of two or three parts: an aglycon base (anthocyanidin), sugars, and possibly acylating groups. Individual anthocyanins are differentiated by their degree of hydroxylation and methylation, and by the position and nature of their glycosylating structures (*3*).

Blueberry (*Vaccinium corymbosum* L.) of the family Ericaceae is reported to have a high content of anthocyanins (*4*, *5*); the effects of cultural systems, organic or conventional, on anthocyanin content were studied (*6*). Anthocyanins are not stable; they are prone to degradation (*7*). After harvest, these compounds undergo changes during processing and storage (*8*, *9*). The native enzyme polyphenol oxidase (PPO), which is present in blueberry, is considered the major enzyme responsible for anthocyanin degradation in fruits and vegetables (*10*) and affects the color of the extract/juice/concentrate. Heating was shown to ensure inactivation of degradation enzymes (*10*). The significant deterioration of phenolic compounds in highbush blueberries when converted to juice has already been discussed in several reports (*11*, *12*). Oxygen, pH, and various storage conditions are shown to have marked effects on anthocyanin stability (*13*). Previous reports are available on pomegranate juice color and bioactive compounds during storage (*14*). Changes in antioxidant capacity or antiproliferation activity due to storage of anthocyanin extracts are also reported (*15*).

Anthocyanins are sensitive to temperature, especially above 70 °C. Kinetic parameters for degradation of anthocyanins were estimated (*16*). Anthocyanin obtained from concord grape was processed, and it was found that the pigment loss, analyzed using spectrophotometry, was 32% at 77 °C, 53% at 99 °C, and 87% at 121 °C (*17*). Degradation of anthocyanins was reported while processing blueberries into juice and concentrate. The rate of degradation of anthocyanins is time and temperature dependent (*18*). Temperature dependence of the degradation of anthocyanins has been shown to follow first-order kinetics (*19*, *20*) and can be modeled using the Arrhenius relationship (*21*). The degradation kinetics of anthocyanins in blood orange juice was studied (*20*). Although many studies have determined the thermal degradation kinetics of anthocyanins in extracts and model systems, very few studies have investigated anthocyanin degradation in solids. Extrusion of corn meal (extruder die temperature 130 °C) with grape juice concentrate and blueberry concentrate showed up to 74% anthocyanin degradation in the extruded cereal (*22*). Extrusion of corn meal with dehydrated blueberry powder in proportions of 84.3%, (cooking temperature up to 130 °C) showed up to 90% decrease of anthocyanins (*22*).

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 Published on Web 10/21/2009 © 2009 American Chemical Society

The potential health benefits of anthocyanins and its bittersweet taste enhance consumer interest in using bluberries as an ingredient in stuffed foods such as fish and in jams. However, information on their degradation in those conditions is not well documented, since there is no study available providing information on fate of anthocyanins and anthocyanidins in blueberry fruit when submitted to heat, for example, when whole blueberries are used in stuffed foods such as fish or when they are used to prepare jams. Nevertheless, the rate of anthocyanin degradation is associated with the rate at which the sugar is degraded to furfural-type compounds derived from the Maillard reaction (*23*). The aim of the present work was to investigate the degradation of anthocyanins and anthocyanidins in blueberry jams and whole blueberries cooked in stuffed foods; for that purpose, the cultivar Bluecrop was chosen.

2. MATERIALS AND METHODS

Materials. The methanol (LiChrosolv), hydrochloric acid and formic acid (purity 98−100%) were provided by Merck (Darmstadt, Germany). Cyanidin 3-*O*-glucoside was from Extrasynthése (Genay, France). Cyanidin, delphinidin, peonidin, and malvidin standards were purchased from Fluka Analytical (Oakville, Ontario, Canada). Stock standard solutions of 1.0 mg/mL in methanol were prepared and used for further dilution.

Sampling. Blueberries (*Vaccinium corymbosum* cv. Bluecrop) used in this study were grown at a farm from Sever do Vouga, Portugal, and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Selected berries were randomized and used for the experiments. Samples were frozen, transported to the University of Porto, and stored at −30 °C for further use.

Portions of 200 g of blueberries were mixed with 200 g of sugar and used to prepare homemade jams. Other portions (50 g) of whole blueberries were used to prepare stuffed fish. Blueberry samples (5 g) ground to paste with mortar and mixed with water were used to evaluate the degradation of anthocyanins and anthocyanins at 100 °C.

Extraction and Hydrolysis of Anthocyanins from Fresh Fruits. Samples consisting of 5.0 g of blueberries (B) ground to paste with mortar were weighed, and 90.0 mL of 0.1% (v/v) hydrochloric acid in methanol was added. The obtained mixture was sonicated for 10 min, put to the thermostat, and left for 30 min at 40 °C and periodically stirred. The solution was filtered, volume completed to 100.0 mL with methanol and diluted to 1:5 using methanol acidified with hydrochloric acid. This methanolic solution was used to evaluate anthocyanin composition at $T = 0$ min; additionally, it was used to hydrolyze the flavonoid glycosides to aglycons (*24*); for that purpose 8.5 mL of concentrated hydrochloric acid was added to 25.0 mL of methanolic solution. The flask was wrapped with aluminum foil and flushed with nitrogen for 5 min. The deoxygenated sample was refluxed at 95 °C for 2 h. The hydrolyzed sample was cooled in the dark, filtered through a 0.45 μm filter and diluted to 50 mL with methanol. These extracts were stored in glass vials flushed with nitrogen. A 20 μL aliquot was injected into the HPLC for analysis.

Assays of Degradation of Anthocyanins and Anthocyanidins. 5.0 g of blueberry samples ground to paste with mortar were weighed to a beaker, 20 mL of water was added and the mixture was heated at 100 °C in a water bath during 15, 20, 25 min (B15, B20, B25). Extraction and hydrolysis of anthocyanins was performed as described for fresh fruits.

Two batches of homemade jam were prepared. Portions of 200 g of blueberries were measured into a kettle, 200 g of sugar was added, and the mixture was boiled, with constant stirring, until the mixture reached 102−105 °C or thickened. Sugar aided in gel formation and acted as a preservative in jams with no added pectin. Samples were taken after 15, 20, and 25 min of cooking; these samples were codified as J15, J20 and J25, respectively.

Two batches of two gilthead sea breams (*Sparus aurata*) weighing around 310±22 g were stuffed with 40 g of whole blueberries cultivar Bluecrop and cooked in an oven during 40 min at 200 °C. The temperature achieved in the blueberries was around 100−102 °C. After cooking, blueberries were removed from fish, and these samples were codified as FB. Analysis of anthocyanins and anthocyanidins were performed as described for fresh fruits.

The soluble solid of ground fresh blueberries (B) mixed with water and without water, blueberries cooked (FB) in stuffed fish and jams was measured at 20 °C using an Abbe refractometor.

HPLC Analysis. Separation and quantification of anthocyanins and anthocyanidins were performed by liquid chromatography with diode array (HPLC/DAD). Diode array detection was set at 520 nm. The chromatographic analysis was carried out in an analytical HPLC unit (Jasco, Japan) equipped with one Jasco PU-1580 HPLC pump, a MD 910 multiwavelength detector and a type 7125 Rheodyne injector with a 20 μL loop. The column was a Spherisorb ODS C_{18} column (5 μm; 250 mm length; 4.6 mm internal diameter). The Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was also used. The mobile phase was as follows: solvent A, 10% formic acid; solvent B, formic acid/water/methanol (10:40:50) as previously described (*25*). The linear gradient program was as follows: 0−50 min, 40−80% B in A; 50−55 min, column rinse and re-equilibration. The flow rate was 1.2 mL/min, and separations were carried out at ambient temperature.

Anthocyanin peaks from blueberry fruits were numbered by order of elution, and peak areas were corrected to units of area/g of blueberry dry matter according to fruit total solids, using the following expression:

$$\text{corrected peak area} = \text{peak area}/(\text{sample amount} \times \text{blueberry dry matter}/100)$$

Areas of anthocyanin peaks in heated fruits and in jams were also corrected according to fruit total solids in samples. Corrected areas were used for calculation of percentage of degradation of anthocyanins in blueberry jams and in heated fruits.

Anthocyanidin peak identification in blueberry samples was carried out by comparing retention times and spectra of unknown peaks with reference standards, as well as cochromatography with added standards. The purity of the peaks was also monitored using the diode array purity test system included in the software. The concentrations of anthocyanidins in fresh blueberries, heated fruits and jams were calculated using corresponding anthocyanidin standard calibration curves, except for petunidin because no standard was available. To overcome this problem peonidin was chosen as reference for calibration, because the chromatographic response of the compound is rather similar at wavelengths around 520−530 nm. Concentration of anthocyanidins was further corrected according to blueberry total solids and expressed as mg anthocyanidin/100 g of blueberry dry matter. In the case of jams it was paid attention to the proportion of blueberry total solids and added sugar, 100 g of total solids jam contained 13.8 g of blueberry total solids.

The detection limits (LOD) for anthocyanidins were calculated as the concentration corresponding to three times the background noise of the blank. A total of twelve analyses with two standard solutions in the linear range, one near the upper and another near the lower limits of concentrations, were performed to evaluate relative standard deviation (RSD %) of the method. Thus, a standard solution containing 0.001 mg/mL of each anthocyanidin and a standard solution containing 0.080 mg/mL of of each anthocyanidin were used. Standard solutions of anthocyanidins were stored in darkness at 4 °C and remained stable over 3 months.

Recovery tests were made by spiking samples with anthocyaninidin standards before hydrolysis. The experimental recovery was obtained from difference between 2 measurements (sample and spiked samples), according to the following relationship:

$$\text{recovery \%} = (\text{total analyte found} - \text{analyte originally present}) \times 100/\text{analyte spike}$$

3. RESULTS AND DISCUSSION

Extraction and HPLC Analysis of Anthocyanins and Anthocyanidins from Blueberry Fruits. The most studied methods for separation and quantification of anthocyanins and anthocyanidins are HPLC with diode array detector (HPLC/DAD). The HPLC chromatogram of Bluecrop extract detected in the visible spectral region (520 nm) revealed ten anthocyanins with retention times of 5.6, 6.1, 6.9, 7.6, 8.6, 9.4, 11.7, 12.5, 13.7, 16.7 min (**Figure 1**). Peaks were numbered by order of elution from 1 to 10.

The UV−vis online spectra using a diode array HPLC detector showed that 10 anthocyanins had their λmax between 515 and 535 nm. Anthocyanins 2 and 3 were acylated with aromatic acids

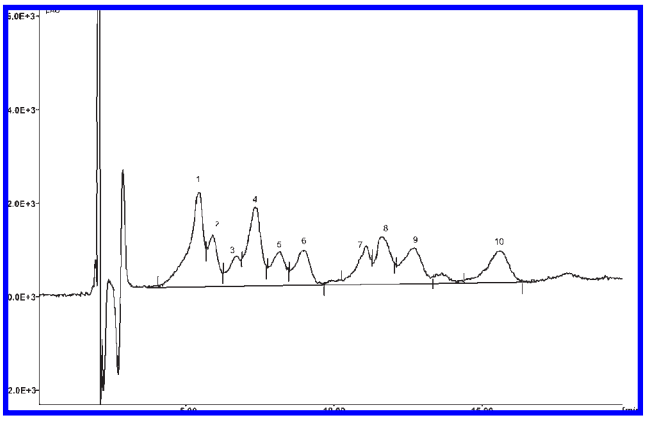

Figure 1. Typical separation of anthocyanins in Bluecrop blueberries using a Spherisorb ODS C_{18} column (5 μm; 250 mm length; 4.6 mm internal diameter) and the chromatographic conditions described in the test. Peaks were numbered by order of elution from 1 to 10.

Table 1. Retention Time, Corrected Area of Anthocyanin Peaks from Bluecrop[a] and Relative Proportion of Each Anthocyanin

peak no.	retention time (min)	corrected peak area	std dev	rel proportion (%)
1	5.68	159723	21087	18.36
2	6.19	55919	1016	6.53
3	6.97	41145	3124	4.46
4	7.64	137994	7318	15.42
5	8.64	63842	2120	6.80
6	9.44	66390	3121	7.09
7	11.77	76172	893	8.89
8	12.48	103990	21241	11.84
9	13.67	87654	1437	10.03
10	16.68	95530	2132	10.59

[a] Expressed as units of area/g of blueberry dry matter.

as evidenced by the presence of peaks in the 300−350 nm region (*26*). Wang et al. (*27*) reported the HPLC analysis of blueberry fruit extracts of both organically and conventionally cultured berries, according to this author's Bluecrop cultivar containing ten anthocyanins, namely, delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside, petunidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-galactoside, and malvidin 3-arabinoside. Our results are in good agreement with those from Wang (*27*), as verified by matching their UV−visible absorption spectrum with its corresponding anthocyanidin standards and relative retention order in the published method.

The chromatograms obtained at a wavelength of 520 nm were used to quantify the anthocyanins. Quantification of anthocyanins in the glycoside form is very difficult because availability of standards for every anthocyanin form present in the sample is poor. Quantification of anthocyanins using HPLC was based mainly on peak areas determined at 520 nm (*28*), which is close to the maximum absorbance wavelength (λmax) of individual anthocyanins and relative proportion of each peak. Area of anthocyanin peaks was corrected according to blueberry dry matter and expressed as units of area/g of blueberry dry matter (**Table 1**).

After acid hydrolysis, the anthocyanidin glycoside pattern can be reduced to anthocyanidins, which are commercially available, except petunidin (*29*). Anthocyanidins are unstable, and precautions must be taken to achieve satisfactory results, avoiding

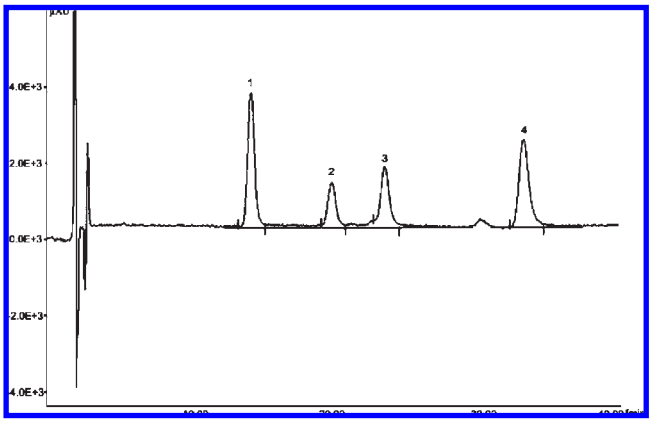

Figure 2. Separation and identification of anthocyanidins in Bluecrop blueberries using a Spherisorb ODS C_{18} column (5 μm; 250 mm length; 4.6 mm internal diameter) and the chromatographic conditions described in the test: 1, delphinidin; 2, cyanidin; 3, petunidin; 4, malvidin.

oxygen and light exposure, and cooling down rapidly after hydrolysis.

Acid hydrolysis of the acidified methanol extract of Bluecrop berries showed four well-separated anthocyanidin peaks with retention times of 14.4, 20.1, 23.7, and 33.5 min. (**Figure 2**). Blueberries contain four of the six common anthocyanidins. The peaks of anthocyanidins were confirmed by matching their retention times and online UV spectral matching to a spectral library made from pure standards, except for petunidin. The match factor typically observed was 95% or greater.

External calibration method was used for quantification of anthocyanidins in hydrolysates of the acidified methanol extract of Bluecrop berries. Linearity was observed in the concentration range of 0.0000561 to 0.010 mg/mL for each anthocyanidin. The coefficients of determination (r^2) were higher than 0.9992. The limit of detection (LOD) was lower than 0.00001 mg, and the RSD values ranged between 1.16 and 8.55%. Detection limits and RSD values for anthocyanidins were similar to those described by Nyman and Kumpulainen (*30*). The reliability of the method was confirmed by two recovery experiments. Recoveries varied between 91.6 and 94.3%. Thus, the aglycons resisted the hydrolysis conditions.

Anthocyanin and anthocyanidin analyses preferably should be performed immediately following extraction or hydrolysis. However, when a great number of samples are assayed, extracts must be preserved until HPLC analysis. Thus, the stability of anthocyanin and anthocyanidin extracts was evaluated at different temperatures and time intervals. For that purpose extracts were stored at −20 and 4 °C in the dark and at 20 °C in the dark and in light in glass vials flushed with nitrogen. The stability of anthocyanin and anthocyanidin extracts was evaluated at 24, 48, and 72 h. Extracts stored in light presented a content of anthocyanins and anthocyanidins significantly lower ($p < 0.05$) then extracts stored in the dark. However, no significant differences were found between anthocyanin and anthocyanidin contents of extracts stored at −20 °C, 4 and 20 °C in the dark ($p > 0.05$).

Degradation of Anthocyanins and Anthocyanidins in Heated Blueberries and in Blueberry Jams. Corrected areas of anthocyanins of Bluecrop fruits (**Table 1**) were used as $T = 0$ min for calculation of degradation of anthocyanins in blueberry samples ground to paste mixed with water (10 °Brix) and heated at 100 °C during 15, 20, and 25 min. Results, expressed as percentage of degradation, are presented in **Figure 3a**. It is clear that the degradation of blueberry anthocyanins increased with heating time. Different susceptibilities of blueberry anthocyanins to heat

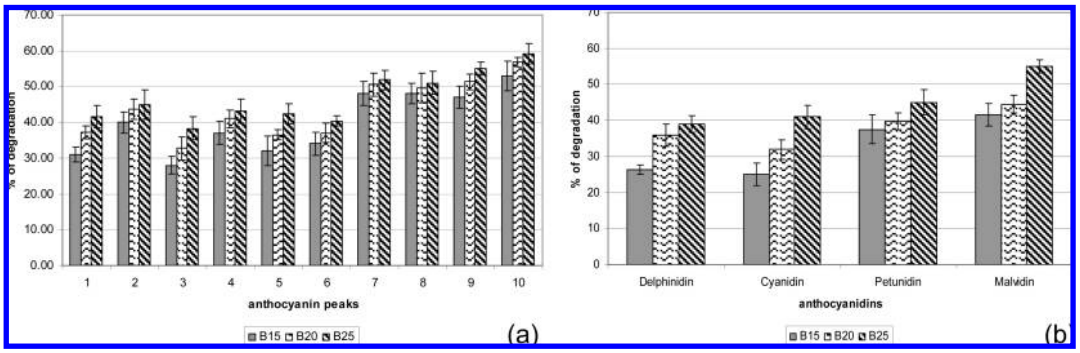

Figure 3. Bar charts showing the percentage of degradation of anthocyanins (**a**) and anthocyanidins (**b**) from Bluecrop fruits ground to paste mixed with water and heated at 100 °C during 15 (B15), 20 (B20) and 25 (B25) min (n = 4). Anthocyanin peaks were numbered from 1 to 10 by order of elution.

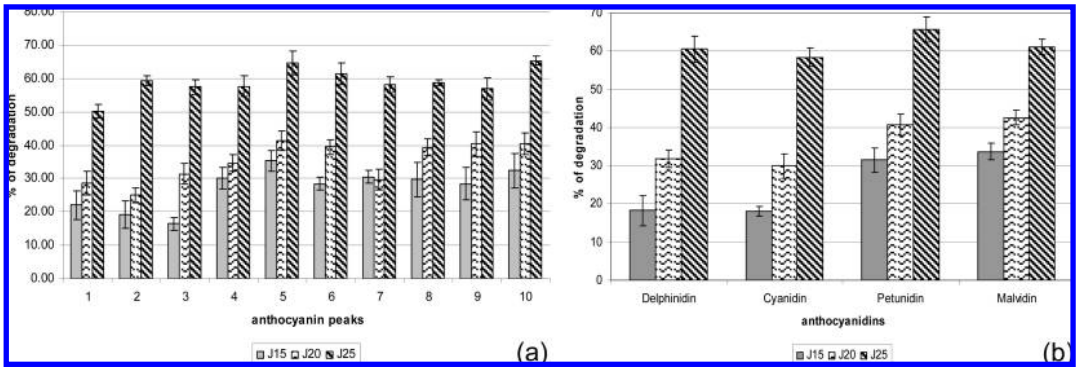

Figure 4. Bar charts showing the percentage of degradation of anthocyanins (**a**) and anthocyanidins (**b**) in Bluecrop jams heated during 15 (J15), 20 (J20) and 25 (J25) minutes (n = 4). Anthocyanin peaks were numbered from 1 to 10 by order of elution.

Table 2. Retention Time and Anthocyanidin Content in Blueberries[a]

peak no.	retention time (min)	peak identification	anthocyanidin concn	std dev
1	14.4	delphinidin	118.6	16.1
2	20.1	cyanidin	124.6	10.8
3	23.7	petunidin	387.8	21.4
4	33.5	malvidin	187.2	7.9

[a] Expressed as mg of anthocyanidin/100 g of blueberry dry matter.

were observed due to their varying anthocyanidin composition. These results are in good agreement with those obtained by other authors for degradation kinetics of anthocyanins in blackberry juice presenting 8.9 °Brix (*31*).

Concerning anthocyanidin degradation, corrected concentrations of anthocyanidins in blueberry fruit expressed as mg of anthocyanidin/100 g of blueberry dry matter (**Table 2**) were used as $T = 0$ min for calculation of degradation of anthocyanidins in heated blueberry samples (**Figure 3b**). The degradation of anthocyanidins presented similar patterns to that observed for anthocyanin degradation.

Results of percentage degradation of anthocyanins in blueberry jams are presented in **Figure 4**. Corrected areas of anthocyanins of Bluecrop fruits (**Table 1**) and corrected areas of anthocyanin peaks in blueberry jams expressed as units of area/g of blueberry dry matter were used to calculated percentage of degradation. As expected, during preparation of blueberry jams anthocyanins suffered degradation. In general, no significant differences ($p < 0.05$) were observed for anthocyanin degradation of J15 and J20, except for peaks 3 and 6. This samples presented °Brix values between 64 and 76. A significant degradation, higher than 50% was observed in anthocyanin peaks of J25 that presented 80 °Brix. It is clear from **Figure 3a** that the thermal degradation of anthocyanins in heated fruits without addition of sugar and mixed with water suffered a different behavior from degradation of anthocyanins in jams (**Figure 4a**). Higher degradation was observed for B15 and B20 and lower degradation was observed for B25 when compared with J15, J20 and J25. Consequently, sugar addition to obtain jam influenced the degradation of anthocyanins. Apparently, for lower heating times (15−20 min) and 64−76 °Brix the addition of sugar had a positive effect on the stability of anthocyanins, however, when the jam presented 80 °Brix, the stability decreased significantly. These results indicate that anthocyanin degradation is associated with the rate at which the sugar is degraded from the Maillard reaction (*23*) and with °Brix of the samples. Wang and Xu (*31*) reported that at higher °Brix anthocyanins were more susceptible to degradation, and the reason might be that the reacting molecules (such as oxygen) become closer, thus the rate of chemical reactions accelerates. According to Rubinskiene et al. (*32*) fructose and glucose were found to be the major sugars in blueberries and the anthocyanin thermostability was influenced by sugar addition.

The degradation of anthocyanidins in jam presented similar patterns to that observed for anthocyanin degradation in jam (**Figure 4b**). Samples with high 80 °Brix presented degradation around 60%.

Changes of Anthocyanins and Anthocyanidins in Cooked Whole Blueberries from Stuffed Fish. Area of anthocyanin peaks in whole blueberries taken from stuffed fish was corrected according to total solids and expressed as units of area/g of blueberry dry matter. Corrected areas of anthocyanins of Bluecrop fruits (**Table 1**) were used for calculation of degradation of anthocyanins in cooked blueberries. Results, expressed as percentage of degradation, are presented in **Figure 5a**. As expected, high degradation of anthocyanins was observed, since the cooking time was 40 min at 100−102 °C. Thus a percentage of degradation between 45 and 50% was not surprising.

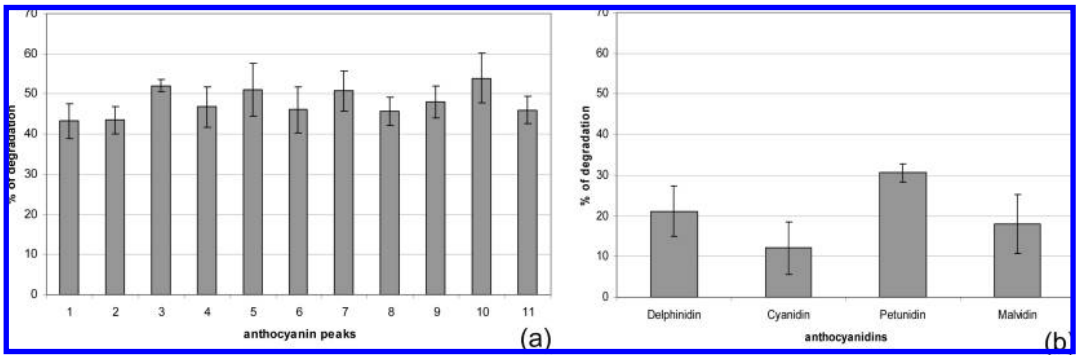

Figure 5. Bar charts showing the percentage of degradation of anthocyanins (**a**) and anthocyanidins (**b**) in cooked whole Bluecrop (n = 8). Anthocyanin peaks were numbered from 1 to 10 by order of elution.

The percentage of degradation of anthocyanidins is presented in **Figure 5b**. Anthocyanins in whole heated fruit suffered lower degradation after 40 min cooking. Delphinidin and malvidin presented degradation around 20%, 30% degradation was observed for petunidin and lower degradation was noticed for cyanidin, 12%. It is known that the composition of anthocyanins varies markedly between the different parts of the blueberry fruit (*33*); the highest content was found in fruit skin, 6223 μg/g, and only 19 μg/g was found in pulp. Anthocyanins heated in whole blueberry inside fish were hydrolyzed to anthocyanidins, but since they were mostly in the berry skin they were protected from light and oxygen, thus, part of the anthocyanidins was not degraded. The generation of anthocyanidins through heating was discussed in a previous study devoted to changes of anthocyanins and anthocyanidins in bilberry extract during dry heating (*34*); about 30% of the degraded anthocyanins was thermally converted to anthocyanidin when the residue of bilberry extract was heated at 100 °C for 30 min. It indicates that anthocyanin could either be broken down to small molecules or suffer loss of its conjugated sugar to become its corresponding anthocyanidin during high heat treatment. This was also observed in cooked whole blueberries from stuffed fish.

In conclusion, anthocyanins and anthocyanidins from blueberries suffered different percentages of degradation at 100 °C depending on several factors, including structure of the compounds, heating time, abundance of oxygen, sugars and their degradation products, and °Brix, among others. Keeping the whole fruit protected from exterior ambient during cooking, such as stuffed fish, can be a good practice to preserve anthocyanidin contents.

LITERATURE CITED

(1) Kay, C. D. Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans. *Nut. Res. Rev*. **2006**, *19*, 137–146.

(2) Seeram, N. P. Berry Fruits for Cancer Prevention: Current Status and Future Prospects. *J. Agric. Food Chem*. **2008**, *56*, 630–635.

(3) Mercadante, A.; Bobbio F. O. Anthocyanins in Foods Occurrence and Physicochemical Properties. In *Food Colorants Chemical and Functional Properties*; Socaciu, C., Ed.; CRC Press LLC: Boca Raton, FL, 2008; pp 241−276.

(4) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in Anthocyanins and Polyphenolics During Juice Processing of Highbush Blueberries (*Vaccinium corymbosum* L.). *J. Food Sci*. **2000**, *65*, 357–364.

(5) Kalt, W.; Ryan, D. A. J.; Duy, J. C; Prior, R. L.; Ehlenfeldt, M. K.; Kloet, S. P. V. Interspecific Variation in Anthocyanins. *J. Agric. Food Chem*. **2001**, *49*, 4761–4767.

(6) Wang, S. Y.; Chen, C.; Sciarappa, W.; Wang, C. Y.; Camp, M. J. Fruit Quality, Antioxidant Capacity, and Flavonoid Content of Organically and Conventionally Grown Blueberries. *J. Agric. Food Chem*. **2008**, *56*, 5788–5794.

(7) Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stability of anthocyanin-based aqueous extract of andean purple corn and red-flushed sweet potato compared to synthetic and natural colourants. *Food Chem*. **2004**, *86*, 69–77.

(8) Kader, F.; Irmouli, M.; Nicolas, J. P.; Metche, M. Involvement of blueberry peroxidase in the mechanisms of anthocyanin degradation in blueberry juice. *J. Food Sci*. **2002**, *67*, 910–915.

(9) Rossi, M.; Giussani, E.; Morelli, R.; Lo Scalzo, R.; Nani, R. C.; Torreggiani, D. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Res. Int*. **2003**, *36*, 999–1005.

(10) Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Mechanism of browning in fresh highbush blueberry fruit (*Vaccinium corymbosum* L). Role of blueberry polyphenol oxidase, chlorogenic acid and anthocyanins. *J. Sci. Food Agric*. **1997**, *74*, 31–34.

(11) Schmidt, B. M.; Eerdman, J. W.; Lila, M. A. Effects of food processing on blueberry antiproliferation and antioxidant activity. *J. Food Sci*. **2005**, *70*, 389–394.

(12) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci*. **2000**, *65*, 357–364.

(13) Kalt, W.; McDonald, J. E.; Donner, H. Anthocyanins, phenolics and antioxidant capacity of processed lowbush blueberry products. *J. Food Sci*. **2000**, *65*, 390–393.

(14) Pérez-Vicente, A.; Serrano, P.; Abellan, P.; García-Viguera, C. Influence of packaging material on pomegranate juice colour and bioactive compounds during storage. *J. Sci. Food Agric*. **2004**, *84*, 639–644.

(15) Srivastava, A.; Akoh, C. C.; Weiguang, Y; Fischer, J.; Krewer, G. Effect of Storage Conditions on the Biological Activity of Phenolic Compounds of Blueberry Extract Packed in Glass Bottles. *J. Agric. Food Chem*. **2007**, *55*, 2705–2713.

(16) Mishra, D. K.; Dolan, K. D.; Yang, L. Confidence Intervals for Modeling Anthocyanin Retention in Grape Pomace during Non-isothermal Heating. *J. Food Sci*. **2008**, 73 Vol. *73*, E9−15.

(17) Sastry, L.; Tischer, R. G. Behavior of the anthocyanin pigments in concord grapes during heat processing and storage. *Food Technol*. **1953**, *6*, 82.

(18) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci*. **2000**, *65*, 357–364.

(19) Cemeroglu, B.; Velioglu, S.; Isik, S. Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *J. Food Sci*. **1994**, *59*, 1216–1218.

(20) Kirca, A.; Cemeroglu, B. Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chem*. **2003**, *81*, 583–587.

(21) Ahmed, J.; Shivhare, U. S.; Raghavan, G. S. V. Thermal degradation kinetics of anthocyanin and visual colour of plum puree. *Eur. Food Res. Technol*. **2004**, *218*, 525–8.

(22) Camire, M. E.; Chaovanalikit, A.; Dougherty, M. P.; Briggs, J. Blueberry and grape anthocyanins as breakfast cereal colorants. *J. Food Sci*. **2002**, *67*, 438–41.

(23) Duhard, V.; Garnier, C.; Megard, D. Comparison of the stability of selected anthocyanin colorants in drink model systems. *Agro Food Ind. Hi-Tech* **1953**, *8*, 28–34.

(24) Burdulis, D.; Ivanauskas, L.; Dirsė, V.; Kazlauskas, S.; Ražukas, A. Study of diversity of anthocyanin composition in bilberry (*Vaccinium myrtillus* L.) fruits. *Medicina* (*Kaunas*) **2007**, *43*, 971–977.
(25) Takeoka, G. R.; Dao, L. T.; Full, G. H.; Wong, R. Y.; Harden, L. A.; Edwards, R. H.; Berrios, J. D. J. Characterization Of Black Bean (*Phaseolus vulgaris L.*) Anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 3395–3400.
(26) Andersen, Ø. M. Chromatographic separation of anthocyanins in Crowberry (Lingonberry) *Vaccinium vites-Idaea* L. *J. Food Sci.* **1985**, *50*, 1230–1232.
(27) Wang, S. Y; Chen, C. T.; Sciarappa, W.; Wang, C. Y.; Camp, M. J. Fruit Quality, Antioxidant Capacity, and Flavonoid Content of Organically and Conventionally Grown. *J. Agric. Food Chem.* **2008**, *56*, 5788–5794.
(28) Gao, L.; Girard, B.; Mazza, G.; Reynolds, A. G. Changes in anthocyanins and color characteristics of Pinot Noir wines during different vinification processes. *J. Agric. Food Chem.* **1997**, *45*, 2003–2008.
(29) Zhang, Z.; Kou, X.; Fugal, K.; McLaughlin, J. Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. *J. Agric. Food Chem.* **2004**, *52*, 688–691.
(30) Nyman, N. A.; Kumpulainen, J. T. Determination of Anthocyanidins in Berries and Red Wine by High-Performance Liquid Chromatography. *J. Agric. Food Chem.* **2001**, *49*, 4183–4187.
(31) Wang, W. D.; Xu, S. Y. Degradation kinetics of anthocyanins in blackberry juice and concentrate. *J. Food Eng.* **2007**, *82*, 271–275.
(32) Rubinskiene, M.; Viskelis, P.; Jasutiene, I.; Viskeliene, R.; Bobinas, C. Impact of various factors on the composition and stability of black currant anthocyanins. *Food Res. Int.* **2005**, *38*, 867–871.
(33) Riihinen, K.; Jaakola, L.; Karenlampi, S.; Hohtola, A. Organ-specific distribution of phenolic compounds in bilberry (Vaccinium myrtillus) and 'northblue' blueberry (Vaccinium corymbosum x V. angustifolium). *Food Chem.* **2008**, *110*, 156–160.
(34) Yue, X.; Xu, Z. Changes of Anthocyanins, Anthocyanidins, and Antioxidant Activity in Bilberry Extract during Dry Heating. *J. Food Sci.* **2008**, *73*, 494–499.

Received for review June 25, 2009. Revised manuscript received September 10, 2009. Accepted October 06, 2009.