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ARTICLE

Cooked Blueberries: Anthocyanin and Anthocyanidin Degradation and Their Radical-Scavenging Activity

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This study examined anthocyanin and anthocyanidin composition and radical-scavenging activity of three cultivars of blueberries (*Vaccinium corymbosum* L., cv. Bluecrop, Bluetravel, and Ozarkblue) before and after cooking. A total of 13 anthocyanins were separated and monitored in methanolic extracts of raw fruits by high-performance liquid chromatography/diode array detector (HPLC/DAD). Principal component analysis using the anthocyanin profile as variables revealed differences according to cultivar origin. Of the six common anthocyanidins, four were identified and quantified in the hydrolysates, namely, malvidin, the most abundant, followed by cyanidin, petunidin, and delphynidin. A systematic evaluation of the degradation of anthocyanins and anthocyanidins of blueberries cooked in stuffed fish was performed. The percentage of anthocyanin degradation in cooked blueberries (by progressive heating from 12 to 99 °C for 60 min) ranged between 16 and 30% for Bluecrop, 30−42% for Bluetravel, and 12−41% for Ozarkblue. However, cooked blueberries maintained or increased radical-scavenging activity when evaluated by the 1,1′-diphenyl-2-picrylhydrazyl (DPPH) method. Overall, results show that cooked blueberries can serve as a good source of bioactive phytochemicals.

KEYWORDS: Cooked blueberries; anthocianins; anthocyanidins; radical-scavenging activity

INTRODUCTION

Oxygen free radicals are involved in many chronic and degenerative diseases, including some types of cancer (*1*−*4*), Alzheimer's disease (*5*), Parkinson's disease, and retina cell damage (*6*). Consequently, modern consumers are aware of the health benefits from eating a diet rich in antioxidants. Red fruits in general, especially blueberries (*Vaccinium corymbosum* L.), are appealing to consumers, owing to their high content of anthocyanins that present radical-scavenging activity (*7*, *8*).

Anthocyanins are glycosylated polyhydroxyl or polymethoxyl derivatives of 2-phenylbenzopyrylium (flavylium) cation and are potent antioxidants *in vitro* (*3*). The pattern of anthocyanins in red fruits is complex, owing to the number and position of hydroxyl and methoxyl groups on the basic anthocyanidin skeleton, the identity, number, and positions at which sugars are attached, the extent of sugar acylation, and the identity of the acylating agent. Fortunately, the complex pattern of anthocyanins can be reduced to six major anthocyanidins by acid hydrolysis. These major aglycones are delphinidin, cyanidin, pelargonidin, petunidin, peonidin, and malvidin (*9*). Only a few papers reported the quantification of anthocynidin (*10*, *11*).

The seasonal availability of antioxidant-rich fruits, market accessibility, cost, and time restraints may limit consumption of fresh fruits on a daily basis. Frozen and cooked products may be selected because of convenience and storage. There is still not enough knowledge about health effects of cooked fruits, and a need for such data still exists.

When fruits are cooked prior to consumption, changes in physical and chemical composition occur compared to the raw form (*11*, *12*). Some studies have investigated the effect of cooking on antioxidant content and activity of foods; however, there are conflicting results (*13*−*17*). For example, recent investigations highlight that thermal degradation of anthocyanins can result in the formation of degradation compounds that also possess antioxidant properties. However, further studies are required to clarify this effect, especially in whole fruits and not modeled systems, which have been studied thus far (*13*). Consequently, cooking protocols should simulate a home kitchen environment using domestic appliances. To better understand the effects of common domestic cooking methods on dietary antioxidant levels obtained by consumers, it is necessary to test real cooking conditions, because the behavior of any food cannot be predicted. Previous studies showed that keeping the whole fruit protected from exterior ambient during cooking, such as stuffed fish, can be a good practice to preserve anthocyanidin contents (*11*). However, these studies have not investigated the effect of degradation of anthocyanins and anthocyanidins in blueberries on their radical-scavenging activity after cooking. Thus, the aim of this study was to compare anthocyanin and anthocyanidin composition of three different cultivars of blueberries and their radical-scavenging activity before and after cooking to investigate if cooked berries can serve as a good source of bioactive phytochemicals.

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MATERIALS AND METHODS

Materials. Methanol (LiChrosolv), hydrochloric acid, formic acid, ascorbic acid, and sodium carbonate all of analytical grade were provided by Merck (Darmstadt, Germany). 1,1′-Diphenyl-2-picrylhydrazyl (DPPH) and rutin were from Sigma-Aldrich Chemical Co. (St. Louis, MO). Cyanidin, delphinidin, peonidin, and malvidin standards were purchased from Fluka Analytical (Oakville, Ontario, Canada).

Sampling. Blueberries (*V. corymbosum* L., cv. Bluecrop, Bluetravel, and Ozarkblue) used in this study were grown at farms 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. For the three cultivars, the sampling collection was performed on the middle of the blueberry harvest period. 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. Samples were coded as BC, Bluecrop; BT, Bluetravel; and OB, Ozarkblue.

Extraction and Hydrolysis of Anthocyanins from Raw Blueberries. A total of 5.0 g of blueberry samples (BC, BT, and OB) ground to paste with mortar were weighted, and 90.0 mL of methanol was added. The obtained mixture was sonicated for 10 min, put to the thermostat, 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 0.1% hydrochloric acid (v/v). This methanolic extract was used to evaluate anthocyanin composition of raw fruits by the spectrophotometrical method and high-performance liquid chromatography/diode array detector (HPLC/DAD); additionally, it was used to hydrolyze the anthocyanins to anthocyanidins (*11*). For that purpose, 25.0 mL of methanolic solution was added to 8.5 mL of concentrated hydrochloric acid. 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 HPLC for analysis. Each sample was analyzed in duplicate.

Quantification of Total Anthocyanins. The total content of anthocyanins was determined by the spectrophotometrical method according to the *European Pharmacopoeia* (*18*). The absorbance of the methanolic extract was measured at 528 nm (Jenway 6105 UV/vis) using a 0.1% (v/v) solution of hydrochloric acid in methanol as the compensation liquid. The content of total anthocyanins was expressed in milligrams of cyanidin-3-*O*-glucoside equivalents (CGEs) per 100 g of blueberry dry matter using a molar extinction coefficient of cyanidin-3-*O*-glucoside of 26 900 mol^{-1} cm^{-1} and molar weight (MW) of 449.2 g mol^{-1}. The dry matter of raw and cooked blueberries was measured at 20 °C using an Abbe refractometor.

Assays of Degradation of Anthocyanins and Anthocyanidins. Two batches of three sea bass fish (*Dicentrarchus labrax* L.) weighing around 379 ± 31 g were stuffed with 56 g of whole blueberries. Each batch contained one fish stuffed with each cultivar Bluecrop, Bluetravel, and Ozarkblue and cooked for 60 min in an oven at 200 °C; the temperature achieved by the blueberries was monitorized using a digital thermocouple (part 0560 9260, Testo 926, Lenzkirch, Germany) with a surface probe (part 0603 1992, Testo 926, Lenzkirch, Germany). After cooking, blueberries were removed from fish and these samples were codified as CBC, CBT, and COB for cooked BC, BT, and OB, respectively. Analysis of anthocyanins and anthocyanidins was performed as described for raw fruits.

HPLC Analysis. Separation and quantification of anthocyanins and anthocyanidins were performed by 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 an ACE C_{18} column (5 μm, 250 mm length, and 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). Two different gradients were used, one for anthocyanin separation and another for anthocyanidin separation.

The linear gradient program used for anthocyanin separation was 0−5 min, 10% B in A; 5−7.5 min, 10−25% B in A keeping this proportion for 5 min; 12.5−30 min, 25−75% B in A; 30−40 min, 75−10% B in A; and 40−45 min, 10% B in A for column rinse and re-equilibration with a flow rate of 1.0 mL/min. Concerning anthocyanidin separation, the linear gradient program used was 0−50 min, 40−80% B in A; and 50−55 min, column rinse and re-equilibration. The flow-rate was 1.2 mL/min, and separations were carried out at room temperature.

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

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

The area of anthocyanin peaks in heated fruits was also corrected according to fruit total solids in samples. Corrected areas were used for calculation of the percentage of degradation of anthocyanins in cooked fruits.

Commercially available anthocyanidin standards were dissolved together in methanol to form a standard mixture of cyanidin chloride, delphinidin chloride, peonidin chloride, and malvidin chloride (1000 mg/L). An external calibration method was used for quantification of anthocyanidins in hydrolysates of the acidified methanol extract of blueberries. The standard mixture was diluted in methanol to obtain diluted standard solutions with concentrations ranging from 0.056 to 10.0 mg/mL. All standard solutions were measured in triplicate. Peak areas of the anthocyanidins in blueberry extracts were within the linear range of the calibration curves. These standards were also used to further confirm the identities of anthocyanidins in the fruit extracts. Because there was no standard available for petunidin, peonidin was chosen as a reference for calibration, because the chromatographic response of the compound is rather similar at wavelengths around 520−530 nm. The concentration of anthocyanidins was further corrected according to blueberry total solids and expressed as milligrams of anthocyanidin per 100 g of blueberry dry matter.

The limits of detection (LODs) for anthocyanidins were calculated as the concentration corresponding to 3 times the background noise of the blank. A total of 12 analyses with two standard solutions in the linear range, one near the upper limit and another near the lower limit of concentrations, were performed to evaluate the 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 each anthocyanidin were used. Standard solutions of anthocyanidins were stored in darkness at 4 °C and remained stable over 3 months.

Radical-Scavenging Activity. Methanolic extracts were prepared for this assay using raw and cooked liophilized blueberries (4 g; 50 mesh) extracted with 100 mL of methanol for 20 min and filtered through a 30 mL borosilicate Robu filter 3.3, pore 4. This procedure was repeated 3 times for each batch. The resulting extracts were combined, and the solvent was evaporated in a rotary evaporator at 40 °C (Büchi Rotavapor RE 111 equipped with a Büchi 461 water bath and Büchi Vac V-500 vacuum pump). Then, the extract was lyophilized and kept in an desiccator in the dark. A dilution series composed of five different concentrations (15.53−1000 μg/mL) of the liophilized extracts was prepared in ethanol at 70 °C using a 96-well plate. The reaction mixtures in the sample wells consisted of 100 μL of methanolic extract and 100 μL of DPPH•, both dissolved in 70% ethanol, prepared daily. The anti-radical activity of the extracts was determined spectrophotometrically in a Biotek ELX808 plate reader (Biotek Corporation, Broadview, IL), by monitoring the disappearance of DPPH• at 515 nm, according to a described procedure (*19*, *20*). The absorbance was measured for 1 h in 1 min intervals, until the reaction reached a plateau. These experiments were performed in duplicate.

The DPPH scavenging effect was expressed as IC_{50}, which is the concentration of extract required to scavenge 50% of the radical present in the reaction medium, and was calculated according to the formula proposed by Sánchez-Moreno et al. (*21*)

$$\text{DPPH scavenging effect (\%)} = 100 - \left(\frac{A_{\text{extract}} - A_{\text{blank 1}}}{A_{\text{control}} - A_{\text{blank 2}}} \times 100\right)$$

where A_{extract} is the absorbance of the extract against the DPPH solution, A_{control} is the absorbance of the DPPH solution, $A_{\text{blank 1}}$ is the absorbance of the extract alone, and $A_{\text{blank 2}}$ is the absorbance of 70% ethanol in the well. The percentage of remaining DPPH against the extract was then plotted to obtain IC_{50}.

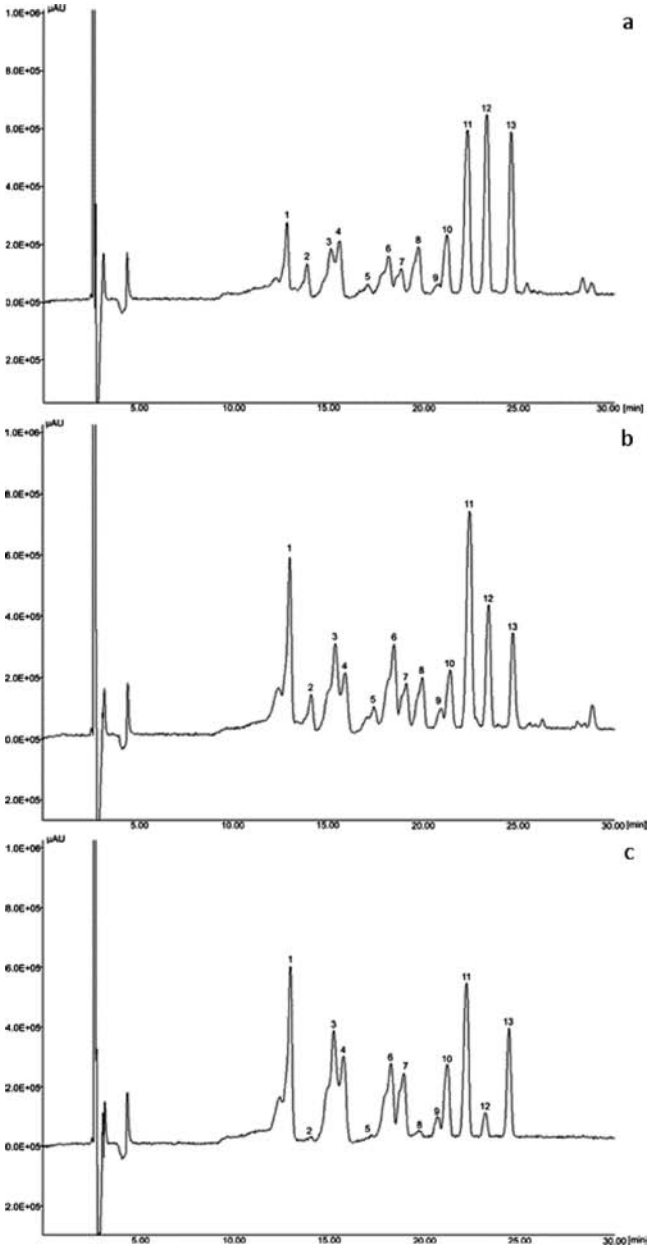

Figure 1. Chromatograms of three cultivars of blueberries: (a) Bluecrop (BC), (b) Bluetravel (BT), and (c) Ozarkblue (OB). Peaks were numbered by order of elution from 1 to 13.

Statistical Analyses. The averages and standard deviations were calculated for each experimental parameter. Descriptive statistics, Student's *t* test and principal component analysis (PCA) were all performed with SPSS for Windows, version 15 (SPSS, Chicago, IL).

3. RESULTS AND DISCUSSION

Anthocyanin Profile in Three Different Cultivars of Raw Blueberries. The HPLC analysis of blueberry raw fruit methanolic extracts revealed that BC and BT cultivars presented similar qualitative patterns when compared to OB. A total of 13 peaks were separated in the first two cultivars; only 10 peaks were separated in the last cultivar (**Figure 1**). Analysis of HPLC chromatograms and peak purity evaluation of mixtures (1:1) of BC and BT, BC and OB, and BT and OB extracts indicated that peaks numbered as 1, 3, 4, 6, 7, 9, 10, 11, 12, and 13 appear in the three cultivars and peaks 2, 5, and 8 were only found in BC and BT cultivars.

Quantification of anthocyanins using HPLC was mainly based on peak areas determined at 520 nm (*5*), which is close to the maximum absorbance wavelength of individual anthocyanins. The area of anthocyanin peaks was corrected according to blueberry dry matter and expressed as units of area per gram of blueberry dry matter. The relative proportion of each peak was also calculated (**Table 1**). The total anthocynin content was higher in BT, when analyzed by HPLC and the spectrophotometric method. Moreover, quantitative differences were observed in a relative proportion of peaks. Major peaks in BC were 11, 12, and 13, whereas major peaks of BT and OB were 1, 3, 6, and 11.

A PCA was performed to simplify the results obtained from the chromatographic separations of anthocyanins. Two new variables were introduced: component 1, which explains approximately 68.6% of the data variance, and component 2, which explains approximately 27.4% of the data variance. The sum of the data variance explained by the two components is approximately 96%. Peaks 1, 3, 4, 6, 7, 9, and 10 were positively associated with component 1, and peaks 2, 5, 8, and 12 were negatively associated with component 1, whereas peaks 6 and 11 were positively correlated to component 2, and peak 13 was negatively correlated with component 2. The results of scores of blueberry cultivars as a function of the two principal components are depicted in **Figure 2**. In this figure, the most important peaks needed for the definition of these components are shown on the edges of the axis, indicating the direction in which the values of these anthocyanins increase, as is conventionally performed in any PCA. As observed in **Figure 2**, it is possible to distinguish three clearly separated groups, each one representing a different cultivar, indicating that the anthocyanin pattern is very useful for the control of product quality, identification of sample cultivar, and examination of consistency in raw materials. The anthocyanin profile as variables was useful for the identification of the cultivar origin and examination of uniformity of raw fruits.

Anthocyanidin Composition of Three Different Cultivars of Raw Blueberries. After acid hydrolysis, the anthocyanidin glycoside pattern can be reduced to anthocyanidins, which are commercially available, except petunidin (*11*). Acid hydrolysis of the acidified methanol extract of the three cultivars showed five well-separated anthocyanidin peaks (**Figure 3**). Delphinidin, cyanidin, and malvidin were identified by matching their retention times and online UV spectral matching to a spectral library made from pure standards. The match factor typically observed was 95% or greater. According to the literature, the other peak was from petunidin (*10*). Additionally, an unknown peak was observed in all samples with a small area and similar retention time of the peonidin standard. However, analysis of the spectra of this peak and the standard does not confirm the identification. Thus, four of the six common anthocyanidins were quantified in blueberries.

Linearity was observed in the concentration range of 0.0561−10.0 mg/L for each anthocyanidin. The coefficients of determination (r^2) were higher than 0.9993. The LOD was lower than 0.01 mg/L, 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 (*10*). **Table 2** gives individual anthocyanidin concentrations in raw fruits of the three cultivars. Malvidin was the most abundant anthocyanidin for the three cultivars, followed by cyanidin, petunidin, and delphynidin.

Anthocyanin and Anthocyanidin Degradation in Cooked Blueberries. The area of anthocyanin peaks in cooked blueberries taken from stuffed fish was corrected according to total solids and

Table 1. Anthocyanin Composition of Three Different Cultivars of Raw Blueberries (Bluecrop, BC; Bluetravel, BT; and Ozarkblue, OB)

		BC		BT		OB	
peak number	retention time (min)	corrected peak area ± SD[a]	RP (%)[b]	corrected peak area ± SD[a]	RP (%)[b]	corrected peak area ± SD[a]	RP (%)[b]
1	13.2	62552 ± 3827	8.4	167882 ± 8305	18.0	182344 ± 7745	21.5
2	14.6	24617 ± 1571	3.3	28045 ± 4273	3.0	nd	0.0
3	15.8	54998 ± 3782	7.4	106758 ± 6872	11.4	151494 ± 7075	17.8
4	16.3	42803 ± 5835	5.7	44302 ± 2742	4.7	68623 ± 4434	8.1
5	18.0	16478 ± 5891	2.2	27526 ± 5760	2.9	nd	0.0
6	18.8	53935 ± 5067	7.2	100444 ± 5185	10.7	101352 ± 4886	11.9
7	19.4	25348 ± 1170	3.4	42768 ± 1642	4.6	69185 ± 2094	8.1
8	20.4	52469 ± 2561	7.0	50976 ± 3249	5.5	nd	0.0
9	21.2	9971 ± 2516	1.3	17947 ± 2889	1.9	18859 ± 599	2.2
10	21.7	49067 ± 919	6.6	44763 ± 2546	4.8	58795 ± 2401	6.9
11	22.7	131989 ± 1579	17.7	165477 ± 3531	17.7	116246 ± 5353	13.7
12	23.7	124128 ± 2264	16.6	79297 ± 2263	8.5	16289 ± 543	1.9
13	25.0	99018 ± 928	13.2	58721 ± 1933	6.3	66859 ± 3119	7.9
TPA[c]		747373		934906		850046	
TAC[d]		1586		1690		1568	

[a] Corrected peak area of anthocyanin ± standard deviation (SD) expressed as units of area per gram of blueberry dry matter. [b] Relative proportion of each anthocyanin expressed as a percentage. [c] Total peak area, obtained by the sum of the corrected area of anthocyanin peaks. [d] Total anthocyanin content, evaluated by the spectrophotometric method and expressed as milligrams of CGEs per 100 g of blueberry dry matter.

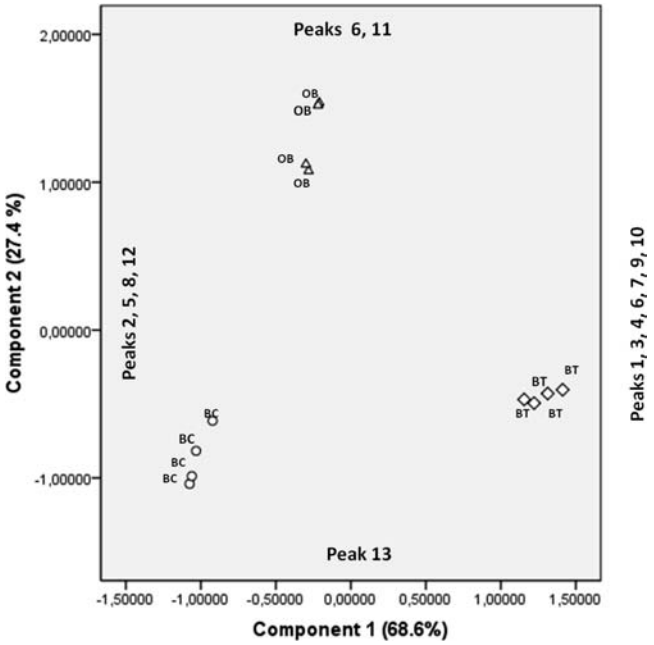

Figure 2. Scores of four batches of three cultivars of blueberry samples (Bluecrop, BC; Bluetravel, BT; and Ozarkblue, OB) on the two principal components.

expressed as units of area per gram of blueberry dry matter. Corrected areas of anthocyanins of blueberry raw fruits (**Table 1**) were used for calculation of the degradation of anthocyanins in cooked blueberries. Results, expressed as a percentage of degradation, are presented in **Figure 4a**. The percentage of anthocyanin degradation ranged between 16 and 30% for CBC, between 30 and 42% for CBT, and between 12 and 41% for COB, indicating some differences between the three cultivars under study. In general, higher degradation was observed in BT samples. It should be pointed out that the monitoring of the blueberry temperature inside fish indicated that it ranged between 12 and 99 °C, but only during the last 20 min, it exceeded 70 °C, because it was 12 °C at $T = 0$, 40 °C at $T = 20$ min, 74 °C at $T = 40$ min, and 99 °C at $T = 60$ min. A previous study using stuffed fish with BC blueberries presents a slightly higher degradation of anthocyanins during a shorter period of time because the

Table 2. Retention Time and Anthocyanidin Content in Three Different Cultivars of Raw Blueberries (Bluecrop, BC; Bluetravel, BT; and Ozarkblue, OB)

			anthocyanidin concentration ± SD[a]		
peak number	retention time (min)	peak identification	BC	BT	OB
1	7.6	delphynidin	12.0 ± 0.6	18.9 ± 0.8	19.6 ± 0.8
2	11.4	cyanidin	111.3 ± 5.8	173.3 ± 6.8	199.0 ± 6.5
3	13.2	petunidin	109.5 ± 2.9	146.7 ± 3.8	135.6 ± 6.4
4	18.5	unknown			
5	20.1	malvidin	1383.8 ± 90.4	1299.8 ± 65.6	1017.4 ± 34.6

[a] Anthocyanidin concentration ± SD, expressed as milligrams of anthocyanidin per 100 g of blueberry dry matter.

blueberries reached 100 °C promptly and were exposed to this temperature most of the cooking time (40 min) (*11*), and in present work, the increase of the temperature was progressive. These results are in agreement with the literature, because anthocyanins are sensitive to the temperature, especially above 70 °C (*22*). Other studies of the influence of the temperature on anthocyanin content of Concord grape analyzed by spectrophotometry indicated a 32% degradation at 77 °C, increasing to 53% at 99 °C (*23*).

The percentage of degradation of anthocyanidins is presented in **Figure 4b**. The anthocyanidins are instable and rarely appear in nature; they have a very short half-life in relation to their glycosylated derivatives. Thus, analysis have been performed by causing the formation of the anthocyanidins by acid hydrolysis of the base material, to enable reliable quantification of the anthocynin aglycones, the molecules responsible for the antoxidant activity. Cyanidin, petunidin, and malvidin presented similar degradation ranging between 20 and 40% degradation. Delphinidin presented lower degradation below 20% for the three cultivars. Thus, it is expected that, after cooking, blueberries retain different amounts of antioxidant activity depending upon the cultivar.

Radical-Scavenging Activity in Cooked Blueberries. The results of the kinetic behavior of blueberry extracts and the amount of antioxidant that is necessary to decrease the initial DPPH concentration by 50% (IC_{50}) of the three cultivars of blueberries before and after cooking are shown in **Figure 5**. After adding the blueberry extract to the DPPH solution, the absorbance increased. The slope of the equations is a useful parameter to define the antioxidant

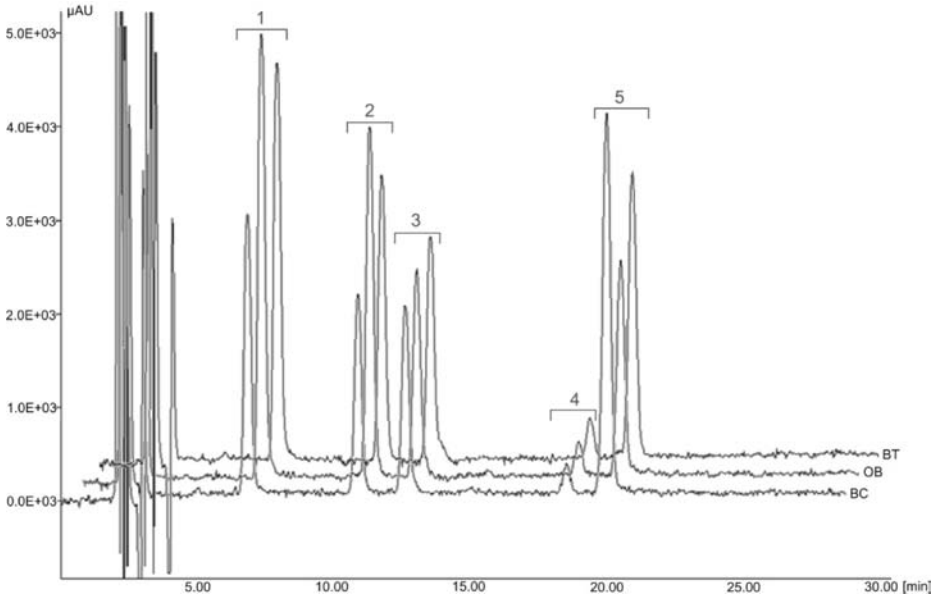

Figure 3. Separation and identification of anthocyanidins in bluecrop (BC), bluetravel (BT) and ozarkblue (OB) blueberries using the chromatographic conditions described in the test: 1, delphinidin; 2, cyanidin; 3, petunidin; 4, unknown; and 5, malvidin.

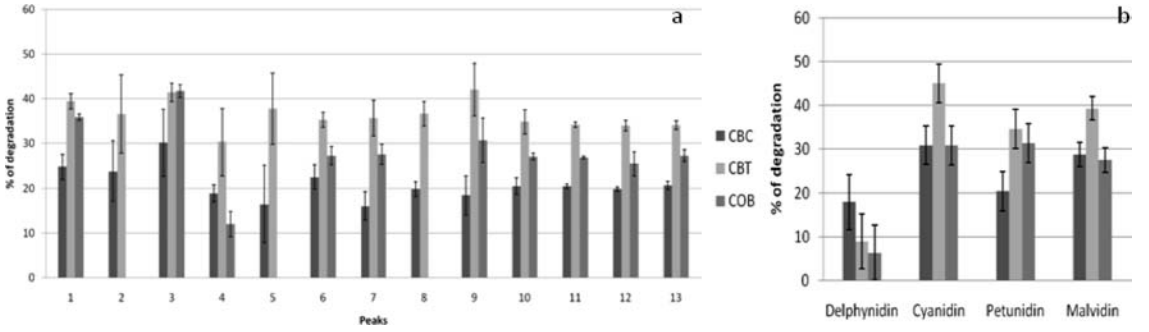

Figure 4. Bar charts showing the percentage of degradation of (a) anthocyanins and (b) anthocyanidins in cooked blueberries. Anthocyanin peaks were numbered from 1 to 13 by order of elution.

activity. The steeper the slope, the lower the amount of antioxidant that is necessary to decrease the initial DPPH concentration by 50% (*19*). With respect to raw blueberries, the steepest slope was that of BT (**Figure 5a**). This means a lower amount of extract was necessary to decrease the initial DPPH concentration (**Figure 5a**). Thus, BT showed a higher antioxidant activity (and also the highest anthocyanin content) then others, while there was no significant difference in the antioxidant effect between BC and OB (nor in the anthocyanin content).

Cooked blueberries (CBC and COB) presented higher antioxidant activity than raw fruits, whereas CBT presented a slight decrease in antioxidant activity, although they were not significantly different (Student's t test, $p > 0.05$). After cooking, the three varieties presented similar radical-scavenging activity.

Anthocyanin degradation is primarily caused by oxidation, cleavage of covalent bonds, or enhanced oxidation reactions because of thermal processing. Thermal degradation of anthocyanins can result in a variety of compounds depending upon the severity and nature of heating (*24*). Adams (*25*) proposed hydrolysis of the sugar moiety and aglycone formation as the initial degradation step possibly because of the formation of cyclic adducts. The author also reported that anthocyanin would decompose upon heating in a chalcone structure, with the latter being further transformed into a coumarin glucoside derivative with a loss of the B ring. A study carried out by Seeram et al. (*26*) demonstrated that high temperatures can cause degradation of cherry anthocyanins, resulting in three different benzoic acid derivatives. However, a separate study conducted by Von Elbe and Schwartz (*27*) suggested that coumarin-3,5-diglycosides are also common thermal degradation products of anthocyanin-3,5-diglycosides.

Some degradation products of anthocyanins from berries were reported to have higher DPPH radical-scavenging activity (*14*, *26*). Especially, degradation products derivate from phenolic acids, whose activity could be comparable to a commercial antioxidant, such as butylated hydroxytoluene (BHT) (*26*). This may support the maintenance or even increase of free-radical-scavenging capability of cooked blueberries. Apparently, the three varieties of blueberries suffered different percentages of degradation of anthocyanins and anthocyanidins after cooking, keeping the radical-scavenging activity for sustainable human health.

The seasonal availability of antioxidant-rich fruits, market accessibility, cost, and time restraints may limit consumption of fresh fruits on a daily basis. However, this study highlights that, although the difference in the relative amounts of anthocyanins and anthocyanidins, the three cultivars of blueberries presented high antioxidant capacity even after cooking. For this reason, the consumption of cooked blueberries is also encouraged as part of a diet rich in fruits, because the antioxidant activity of blueberries was not negatively affected by processing. This information is

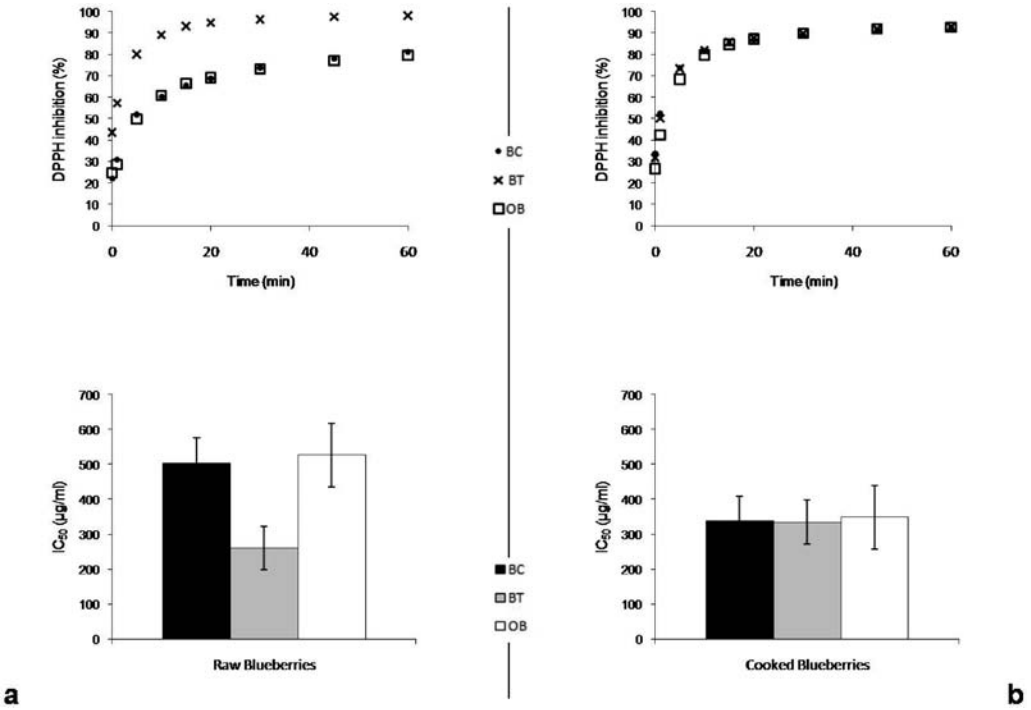

Figure 5. Kinetic behavior of the reducing DPPH radical and bar charts showing IC_{50} for the three cultivars of blueberries (a) before and (b) after cooking.

relevant owing to the increasing consumer and industry awareness concerning the nutritional value of cooked foods.

ABBREVIATIONS USED

BC, Bluecrop; BT, Bluetravel; OB, Ozarkblue; CBC, cooked Bluecrop, CBT, cooked Bluetravel; COB, cooked Ozarkblue.

LITERATURE CITED

(1) Lazz, M. C.; Savio, M.; Pizzala, R.; Cazzalini, O.; Perucca, P.; Scovassi, A. I.; Stivala, L. A.; Bianchi, L. Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. *Carcinogenesis* **2004**, *25*, 1427–1433.

(2) Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003**, *5*, 68–75.

(3) Yi, W.; Fischer, J.; Krewer, G.; Akoh, C. C. Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *J. Agric. Food Chem.* **2005**, *53*, 7320–7329.

(4) Kamei, H.; Kojima, T.; Hasegawa, M.; Koide, T.; Umeda, T.; Yukawa, T.; Terabe, K. Suppression of tumor cell growth by anthocyanins in vitro. *Cancer Invest.* **1995**, *13*, 590–594.

(5) Joseph, J. A.; Denisova, N. A.; Arendash, G.; Gordon, M.; Diamond, D.; Shukitt-Hale, B. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr. Neurosci.* **2003**, *6*, 153–162.

(6) Jang, Y. P.; Zhou, J.; Nakanishi, K.; Sparrow, J. R. Anthocyanins protect against A2E photooxidation and membrane permeabilization in retinal pigment epithelial cells. *Photochem. Photobiol.* **2005**, *81*, 529–536.

(7) Zheng, W.; Wang, S. Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 502–509.

(8) Faria, A.; Oliveira, J.; Neves, P.; Gameiro, P. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *J. Agric. Food Chem.* **2005**, *53*, 6896–6902.

(9) Kahkonen, M. P.; Heinamaki, J.; Ollilainen, V.; Heinonen, M. Berry anthocyanins: Isolation, identification and antioxidant activities. *J. Sci. Food Agric.* **2003**, *83*, 1403–1411.

(10) Nyman, N.; Kumpulainen, J. Determination of anthocyanidins in berries and red wine by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49*, 4183–4187.

(11) Queiroz, F.; Oliveira, C.; Pinho, O.; Ferreira, I. M. P. L. V. O. Degradation of anthocyanins and anthocyanidins in blueberry jams/stuffed fish. *J. Agric. Food Chem.* **2009**, *57*, 10712–10717.

(12) Pellegrini, N.; Chiavano, E.; Gardana, C.; Mazzeo, T.; Contino, D.; Gallo, M.; Riso, P.; Fogliano, V.; Porrini, M. Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen brassica vegetables. *J. Agric. Food Chem.* **2010**, *58*, 4310–4321.

(13) Patra, A.; Nigel, P.; Brunton, N. P.; O'Donnell, C.; Tiwari, B. K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci. Technol.* **2010**, *21*, 3–11.

(14) 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.

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

(16) 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.

(17) 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*, 525–528.

(18) *European Pharmacopoeia*; Council of Europe: Strasburg, France, 2005; pp 1099−1100.

(19) Blois, M. S. Antioxidant determination by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.

(20) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30.

(21) 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.

(22) Mishra, D. K.; Dolan, K. D.; Yang, L. Confidence intervals for modeling anthocyanin retention in grape pomace during nonisothermal heating. *J. Food Sci.* **2008**, *73*, 9–15.

(23) Sastry, L.; Tischer, R. G. Behavior of the anthocyanin pigments in concord grapes during heat processing and storage. *Food Technol.* **1953**, *6*, 82–85.

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

(25) Adams, J. B. Thermal degradation of anthocyanin with particular reference on 3 glucosides of cyanidin. In acidified aqueous solution at 100 °C. *J. Sci. Food Agric*. **1973**, *24*, 747–762.

(26) Seeram, N. P.; Bourquin, L. D.; Nair, M. G. Degradation products of cyanidin glycosides from tart cherries and theirbioactivities. *J. Agric. Food Chem*. **2001**, *49*, 4924–4929.

(27) Von Elbe, J. H.; Schwartz, S. J. Colorants. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker, Inc.: New York, 1996; pp 651−722.

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