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ARTICLE

Pressure and Temperature Effects on Degradation Kinetics and Storage Stability of Total Anthocyanins in Blueberry Juice

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The degradation kinetics of total anthocyanins in blueberry (*Vaccinium myrtillus*) juice were studied during thermal processing by treatment at selected temperatures (60−121 °C) and combined high pressure−temperature processing (100−700 MPa, 40−121 °C). Anthocyanin stability was also studied for several of these treatments during storage at 4, 25, and 40 °C. Both pressure and temperature increased *d*, the degradation rate of total anthocyanins in blueberry juice, meaning that at constant temperature, anthocyanins were more rapidly degraded with increasing pressure. For example, 32% degradation of anthocyanins was observed after 20 min heating at 100 °C and atmospheric pressure, whereas at 100 °C and 600 MPa, approximately 50% of total anthocyanins were lost. Degradation of anthocyanins was significantly accelerated with increasing storage temperatures. Combined pressure−temperature treatment of pasteurized juice led to a slightly faster degradation of total anthocyanins during storage compared to heat treatments at ambient pressure. Degradation of anthocyanins was best described by a 1.4th-order reaction at all conditions investigated. A mathematical model describing the degradation of blueberry anthocyanins in juice as a function of pressure, temperature, and treatment time is presented.

KEYWORDS: Total anthocyanins; blueberry juice; high pressure; degradation kinetics; storage stability

INTRODUCTION

The nutritional quality of processed foods is of great interest to the consumer and food processing industry due to its direct and indirect impact on consumer's health. Blueberries are a rich dietary source of different phytonutrients including anthocyanins that significantly contribute to its antioxidant capacity (*1*). Anthocyanins are naturally occurring phenolic compounds responsible for the attractive colors of many fruits and vegetables and are believed to reduce the risk of heart and other chronic diseases (*2*). Blueberries are often processed into juices, smoothies, or concentrates for direct consumption or to be used as natural food ingredients and colorants. Upon processing, blueberry anthocyanins can be quickly oxidized presumably due to the presence of native polyphenol oxidases (PPO) (*3*, *4*). However, some anthocyanins are not good substrates for PPO due to their structure. The sugar moiety of anthocyanins is believed to be a steric hindrance against PPO attack. The removal of the sugar moiety by the action β-glucosidase results in the formation of anthocyanidins which then can be more easily oxidized by PPO (*5*). PPO also catalyzes the oxidation of blueberry monophenols and hydroxycinnamic acid derivatives to *o*-diphenols and highly reactive *o*-quinones, which react with anthocyanins to produce brown degradation products (*5*, *6*).

Oxidative enzymes and spoilage organisms in fruit and vegetable products are usually controlled by thermal blanching at temperatures above 80 °C, which also proved to be effective in eliminating PPO and significantly increasing the recovery and stability of anthocyanins in blueberry juice (*4*). However, at these process conditions, blueberry products typically lose their natural flavor and some of their nutritional value. The storage stability of blackberry anthocyanin pigments is also reported to be affected after exposure to long treatment times at high temperatures (*7*).

High pressure processing (HPP) can inactivate vegetative cells of microorganisms and some enzymes near room temperature, making it a suitable pasteurization method for foods high in heat labile bioactive or volatile compounds (*8*). HPP of juices sufficiently kills spoilage organisms and reduces PPO activity (*9*), while anthocyanin pigments are only minimally affected (*10*).

Patras et al. (*11*) reported no significant change in anthocyanin content of strawberry and blackberry purées after 15 min treatments at 500−600 MPa near room temperature. Similarly, cyanidin-3-glucoside, a predominant anthocyanin in berries, was found to be stable in a model solution at 600 MPa and 20 °C for up to 30 min (*12*). However, 30 min application of 600 MPa at 70 °C reduced cyanidin-3-glucoside by 25%, whereas only 5% was lost after 30 min heating at the same temperature and

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ambient pressure, indicating that pressure can accelerate anthocyanin degradation at elevated temperatures. On the other hand, the degradation of anthocyanins in a food matrix (red grape extract solutions) was negligible after a combined treatment at 600 MPa and 70 °C during 60 min (*13*).

Fruit juices made from rasperries, strawberries, black currants, and muscadine grape showed greater storage stability of anthocyanins after 15 min exposure to high pressures (500−800 MPa) at room temperature compared to control juices (*14*−*17*). There is some evidence that this stability increase is due to the reduced activity of PPO or β-glucosidase in the pressurized juice (*17*, *18*). However, PPO activity can also increase in fruit juices upon exposure to specific pressures (*9*), and there have been reports that the pressure stability of PPO in strawberry or apple tissue can exceed the current economical limit for high pressure processing of foods of up to 700 MPa at room temperature (*19*, *20*).

Pressure application at elevated temperatures has been investigated as a potential alternative to conventional thermal sterilization for ambient shelf stable products, in part because of its ability to efficiently inactivate bacterial and fungal endospores (*21*). Often the required inactivation temperature and/or time is lowered in combination with pressure, which can enhance the retention of nutrients and color components of the processed food (*22*). Little is known about the impact of high pressure applied at elevated temperature on the stability of anthocyanins in foods. However, the process conditions governing the rate of anthocyanin degradation need to be understood to ensure the best possible recovery during processing and storage of blueberry products. Hence, the objective of this study was to study the effects of combined pressure−temperature treatments on retention and storage stability of anthocyanins in blueberry juice.

MATERIALS AND METHODS

Blueberry Juice Extraction. Frozen highbush blueberries (*Vaccinium myrtillus*, Blue Rose) obtained from a local grower were thawed, washed, and blended using a Waring blender (no. 700S, Waring laboratory, Torrington, CT). The purée was pasteurized at 85 °C for 5 min in a water bath to disrupt the cell structure and to inactivate anthocyanin degrading enzymes. No heat was applied for untreated juice production. The juice was separated from the pulp by centrifugation (J2-MC, Beckmann, CA) at 12000*g* for 30 min at 4 °C. After filtration through glass wool, the juice was filled into glass tubes (Alltech Associates Inc., Deerfield, IL). The juice was stored frozen at −18 °C until further use. The soluble solid content (°Brix) of the juice was measured at 20 °C using an Abbe refractometor (Atago, Tokyo, Japan). Titratable acidity was determined according to the standard AOAC method (AOAC, 2003).

Thermal and High Pressure Treatment. Pasteurized (enzyme inactivated) blueberry juice was treated at temperatures from 40 to 121 °C and 0.1−700 MPa under isobaric/isothermal conditions. For studying the thermal degradation kinetics of anthocyanins, blueberry juice was filled in 100 μL glass capillaries (Brand, Wertheim, Germany), which were heat sealed with a Bunsen burner. The capillaries were immersed either in a thermostatted water-bath or oil-bath at temperatures ranging from 60 to 121 °C for selected residence times. Following treatment, the samples were immediately cooled in iced-water. The anthocyanin content of the samples was measured within 4 h after treatment. The initial anthocyanin concentration (C_0) was defined as the concentration found after heating the glass capillary to the target temperature (approximately 3 s) followed by immediate cooling of the sample in iced-water.

Anthocyanin degradation kinetics under high pressure conditions was investigated using a laboratory scale high-pressure equipment (no. U111, Unipress, Warsaw, Poland) as described previously (*23*). The target temperature was varied between 40 and 121 °C, and the pressure ranged from 100 to 700 MPa. Thawed blueberry juice was transferred into 1.5 mL flexible cryo tubes (no. 5000-1012, Nalgene, Rochester, NY) and placed into the pressure vessels preheated to the target temperatures. Pressurization was started when the sample reached a temperature level which would result in the target temperature after compression heating. The sample temperature was measured with a thermocouple, which was placed directly inside the tubes. The compression rate was standardized at approximately 20 MPa s^{-1} to minimize anthocyanin loss during the pressure build-up phase. The initial anthocyanin concentration (C_0) was defined as the concentration found immediately after pressure build-up. After pressure release, the samples were stored in ice−water, and the anthocyanin content was measured within 4 h after the treatment. All experiments were performed in duplicate.

Storage Pretreatment. The stability of anthocyanins in untreated, pasteurized (purée heated at 5 min at 85 °C), heat treated, and pressurized blueberry juice was studied during storage for up to 42 days in the dark in controlled temperature incubation cabinets maintained at 4, 25, and 40 °C. Sampling times depended on the pretreatment and storage conditions and ranged between 1 and 7 days. Screw-cap glass vials (1.4 mL, Alltech Associates Inc., Deerfield, IL) were completely filled (no headspace) with aliquots of untreated or pasteurized blueberry juice. The heat treated and the pressurized juices were filled in cryovials (Nalgene, Rochester, NY) and were subjected to thermal treatments at 90 and 110 °C for 5 min (excluding 3 min temperature come-up time) in a thermostatted oil-bath or high pressure treatment at 600 MPa and 70, 90, and 110 °C for 5 min, respectively. The treated samples were cooled in iced-water for approximately 1 h before the juice was transferred into screw-cap glass vials.

Anthocyanin Assay. Total monomeric anthocyanin content of the juice was determined using the pH differential method (*24*). Blueberry juice was diluted 1:20 in 25 mM potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5, respectively. The absorbance of the solutions was measured spectrophotometrically at 510 and 700 nm (UV-1700 Pharma Spec, Shimadzu, Tokyo, Japan). The absorbance difference A was calculated as:

$$A = (A_{510} - A_{700})\text{pH1.0} - (A_{510} - A_{700})\text{pH4.5} \quad (1)$$

The total anthocyanin concentration C (mg L^{-1}) was expressed as mg cyanidin-3-glucoside equivalents according to the following equation:

$$C = A \cdot \text{MW} \cdot \text{DF} \cdot \frac{1000}{(\varepsilon \cdot l)} \quad (2)$$

With MW, Molecular weight for cyanidin-3-glucoside (449.2 g mol^{-1}); DF, dilution factor; l, path-length in cm; ε, molar extinction coefficient for cyanidin-3-glucoside (26900 L mol^{-1} cm^{-1}); 1000, conversion from g to mg. All analyses were performed in triplicate.

Total Phenolic Content. The concentration of total phenols in the extracts was measured according to the Folin−Ciocalteu method (*25*). Gallic acid (no. G7384, Sigma-Aldrich) was used as a standard. Twenty μL of the juice was added to 1.58 mL of water and 100 μL of Folin−Ciocalteu reagent (no. F9252, Sigma-Aldrich). After 3 min, 300 μL of Na_2CO_3 reagent (75 g L^{-1}) was added. The samples were then incubated for a total of 30 min at room temperature, and the absorbance of the sample was read at 765 nm against a blank. The value was converted to gallic acid equivalent using a standard curve.

Enzyme Assays. The extraction solution for all enzyme assays was 0.2 M sodium phosphate buffer (pH 6.5) containing 4% (w/v) poly(vinylpolypyrrolidone) (PVPP) (no. 057K0001, Sigma-Aldrich) and 1% (v/v) Triton X-100 (no. T9284, Sigma-Aldrich). Ten mL of juice was mixed with 20 mL of the enzyme extraction solution. The mixture was homogenized under ice cooled condition for 3 min and subsequently centrifuged (no. J2-MC, Beckmann, CA) at 14000*g* for 30 min at 4 °C. The supernatant was used for the enzyme assays.

Peroxidase (POD) and polyphenol oxidase (PPO) activities were determined by following the procedure described previously (*19*). One unit of POD or PPO was defined as the observed change in absorbance at 40 °C per mL of blueberry juice.

The β-glucosidase assay was modified from Hamon et al. (*26*). Briefly, 100 μL of the enzyme extract was added to 500 μL of 40 mM *p*-nitrophenyl-β-D-glucopyranoside (no. N7006, Sigma-Aldrich) solution and 400 μL of 0.1 M citrate-phosphate buffer (pH 4). The reaction mixture was incubated for 30 min at 40 °C. Then 2 mL of 3 M sodium carbonate solution was added to stop the reaction. The *p*-nitrophenol formed was measured at 405 nm and 20 °C against a blank. The concentration of the product was calculated from the extinction coefficient, ε = 18350 $M^{-1}cm^{-1}$,

obtained from a standard curve of *p*-nitrophenol. One unit of β-glucosidase activity was defined as the amount of enzyme catalyzing the hydrolysis of 1 μmol of *p*-nitrophenyl-β-D-glucopyranoside/min.

Kinetic Data Analysis. Thermal degradation of anthocyanins has often been expressed by a simple first-order reaction (*27*):

$$\frac{dC}{dt} = -kC \tag{3}$$

where t is the time (min), k is the rate constant (min^{-1}), and C is the anthocyanin concentration at time t (mg mL^{-1}).

The rate constant k at different pressure−temperature combinations can be obtained by regression fitting the data to eq 3 using a statistical software (Table Curve 2D v4.0 Statistical Package, Systat Software Inc., Richmond, CA).

The half-life $t_{1/2}$ (days) of anthocyanins during storage was expressed by the following equation:

$$t_{1/2} = -\frac{\ln(0.5)}{k} \tag{4}$$

In the cases where deviations from simple first-order reactions are observed at isobaric and isothermal conditions, the *n*th-order reaction model may be more appropriate (eq 5). Because the anthocyanin degradation kinetics data showed some deviation from simple first-order kinetics, the data were fitted to an *n*th-order reaction model (eq 5). Integration of eq 5 yields eq 6, introducing the specific inactivation rate constant k', which is dependent on the initial anthocyanin concentration C_0 (eq 7) (*9*).

$$\frac{dC}{dt} = -kC^n \tag{5}$$

$$\frac{C}{C_0} = \frac{1}{1-n}(1 + k' \cdot t \cdot (n-1)) \tag{6}$$

$$k' = (k \cdot C_0^{(n-1)}) \tag{7}$$

where C_0 represents the initial anthocyanin concentration (mg mL^{-1}) and n is the reaction order.

Initially, the kinetic data were fitted to eq 6 with reaction orders ranging from 1 to 1.8. The reaction order n, which provided the smallest cumulative minimum of the fit standard error of fit, was chosen in order to obtain a single reaction order best matching the kinetics data of all pressure−temperature conditions. After fixing the reaction order, the specific inactivation rate constants were regressively obtained by fitting the data to eq 6. C_0 was assumed to be constant in all experiments. This was done to obtain comparable values of the inactivation rate, which can then be used for secondary modeling (*9*).

Modeling Anthocyanin Degradation at Isothermal/Isobaric Conditions. To facilitate comparison with other work, the dependence of the calculated first-order rate constants on temperature at a constant pressure was expressed by the activation energy E_a (kJ mol^{-1}), which was calculated from the Arrhenius equation:

$$k = k_{ref} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{8}$$

where k_{ref} (min^{-1}) is the degradation rate constant at the reference temperature T_{ref}, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is absolute temperature (K).

The activation volume $\Delta V^{\#}$ (cm^3 mol^{-1}), as a measure for the pressure dependence of k, can be estimated using the Eyring relationship:

$$k = k_{ref} \exp\left[-\frac{\Delta V}{RT}(p - p_{ref})\right] \tag{9}$$

where p is the pressure (MPa), and k_{ref} (min^{-1}) is the degradation rate constant at the reference pressure (p_{ref}). Both $\Delta V^{\#}$ and E_a can be estimated by linear regression analysis of the natural logarithm of the k values versus the pressure or the reciprocal of the absolute temperature, respectively.

The effect of pressure and temperature on the specific rate constant k' was expressed using a thermodynamically based third-order polynomial equation (eq 10), which has been successfully used to describe the degradation of food compounds as function of pressure and temperature (*20*):

$$\begin{aligned}\ln(k') = {} & A + B\cdot(p-p_0) + C\cdot(T-T_0) + D\cdot(p-p_0)^2 + E\cdot(T-T_0)^2 \\ & + F\cdot(p-p_0)\cdot(T-T_0) + G\cdot(p-p_0)^3 + H\cdot(T-T_0)^3 \\ & + I\cdot(p-p_0)^2\cdot(T-T_0) + J\cdot(p-p_0)\cdot(T-T_0)^2\end{aligned} \tag{10}$$

The parameters A, B, C, D, E, F, G, H, I, and J were estimated by nonlinear regression analysis of the estimated k' values at different pressure−temperature combinations using a statistical program (Table Curve 3D v3 Statistical Package, Systat Software Inc., Richmond, CA). The significance of the model parameters used in eq 10 was assessed by the mean square error (MSE).

$$\mathrm{MSE} = \frac{\sum(k_{pred} - k_{obs})^2}{N - P} \tag{11}$$

where N is the number of degradation rate constants used for the model, P is the number of model parameters, k'_{pred} is the predicted specific rate constant and k'_{obs} is the observed specific rate constant at a given $p-T$ combination. The MSE is given by the difference between the observed and the predicted values by the model and is often used in statistics to determine the degree of fit of the model to the data and whether the model can be simplified by removing terms. Typically, the smaller the MSE values, the better the fit of the model to the data. Hence, starting from eq 10, third- and second-order model parameters were eliminated until a minimum in MSE was found.

The performance of the secondary model (eq 10) was also assessed by calculating the accuracy factor (A_f) (*28*), which is a simple multiplicative factor indicating the spread of results around the prediction:

$$A_f = \exp\left[\frac{1}{N}\sum_{i=1}^{N}\left(\log\left(\frac{k_{pred}}{k_{obs}}\right)\right)^2\right]^{(\ln 10)/2} \tag{12}$$

The smaller the A_f value, the more accurate are the absolute errors in the model. A value of 1 indicates the perfect agreement of the model to the data fitted.

RESULTS AND DISCUSSION

Juice Characteristics. Table 1 shows some characteristics of the blueberry juice before and after pasteurization. The pasteurized juice contained 229 and 848 mg L^{-1} of total anthocyanins and phenolics, respectively. This is within the reported range of 166−448 mg L^{-1} anthocyanins (*3*, *4*, *29*, *30*) and 686−3510 mg L^{-1} phenolics (*29*,*30*) in pasteurized blueberry juice, depending on the source and processing conditions. The untreated juice showed lower content of anthocyanins and phenolics than the pasteurized juice, possibly due to the presence of anthocyanin degrading enzymes and a lower proportion of disintegrated cells. This is in accordance with other reports on increased anthocyanin and phenolic yields in the juice after heat pretreatments of blueberry purée (*29*, *30*). The untreated blueberry juice showed significant activity of PPO and β-glucosidase and considerable activity of POD. These enzymes are known to directly or indirectly degrade anthocyanins (*18*), but they were inactivated below our detection limit after a 5 min heat treatment of the blueberry purée at 85 °C (**Table 1**).

Thermal Stability of Anthocyanins. Figure 1A shows isothermal degradation kinetics of anthocyanins in pasteurized blueberry juice at ambient pressure between 70 and 121 °C. Blueberry anthocyanins were relatively stable in the juice up to 60 °C, showing less than 10% degradation within 120 min heating time (results not shown). As expected, anthocyanin degradation was accelerated at higher temperatures. For example, incubation at 80, 90, 100, 110, and 115 °C resulted in 50% degradation of

Table 1. Characteristics of Untreated and Pasteurized Blueberry Juice

	untreated juice	pasteurized juice
°Brix	12.50 ± 0.05[a]	12.70 ± 0.05
pH	3.30 ± 0.02	3.00 ± 0.02
titration acidity (mg mL^{-1})[b]	705 ± 6	791 ± 5
total anthocyanines (mg L^{-1})[c]	152 ± 16	229 ± 8
total phenolics (mg L^{-1})[d]	626 ± 20	848 ± 18
polyphenoloxidase (U mL^{-1})	46.7 ± 4.5	<1.0
peroxidase (U mL^{-1})	5.1 ± 0.1	<0.1
β-glucosidase (U L^{-1})	459 ± 14	<9

[a] Standard deviation. [b] Expressed as mg of anhydrous citric acid per mL. [c] Expressed as mg of cyanidin-3-glucoside equivalents per L. [d] Expressed as mg of gallic acid equivalents per L.

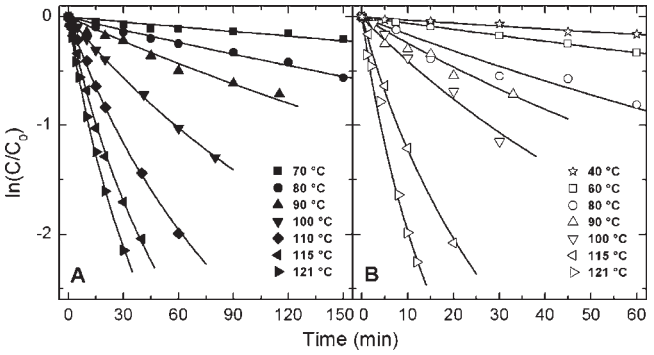

Figure 1. Thermal degradation kinetics of total anthocyanins in pasteurized blueberry juice at ambient pressure (**A**) and at 600 MPa (**B**). The lines interpolating the experimental data points show the fit of a 1.4th-order reaction model (eq 6).

anthocyanins after approximately 180 (data point not shown), 115, 40, 15, and 10 min, respectively. This half-life is in the same order of magnitude as reported for the thermal stability of grape malvidin-3-glucoside in citrate buffer (pH 3.5) (*31*) and anthocyanin degradation in blueberry, blackberry (*32*), and blood orange juice (*33*, *34*). On the other hand, anthocyanins were more susceptible to high temperatures in plum purée (*35*) and showed higher thermal stability in sour cherry (*36*) and blackberry juice (*7*, *37*) and in extracts of red-flesh potato (*38*) and black carrots (*39*).

Different heat stabilities of fruit juice anthocyanins may be explained by the variation in the heat stability of different fruit anthocyanins (*31*). The major anthocyanins in blueberries are malvidin- and delphinidin-3-glycosides with smaller quantities of peonidin- and petunidin-3-glycosides (*3*, *40*) whereas, for example, blackberries largely contain cyanidin-3-glucosides (*41*, *42*). Delphinidin glycosides exhibit a greater temperature liability due to their three ortho phenolic groups in the B ring in comparison to cyanidin derivatives, which only have two ortho phenolic groups and, thus, a second-order of reactivity (*3*). The thermal stability of anthocyanins is also reduced by the number of hydroxyl groups on the A ring in the absence of the dihydroxy structure in the B ring and the degree of glycosylation in the 3 position; however, the correlation between anthocyanin stability and chemical structure is still far from clear (*43*).

Anthocyanin stability at high temperatures is also known to be largely affected by a number of environmental factors such as pH (*39*, *44*) and the presence of anthocyanin degrading enzymes (*6*), ascorbic acid, and/or free radicals (*15*, *45*) or oxygen (*27*), which are likely to differ depending on fruit origin and processing conditions. Low acidity (i.e., pH > 3) has been shown to considerably reduce the thermal stability of anthocyanins in different media (*31*, *44*). Hence, it is not surprising that the thermal stability of anthocyanins was higher in sour cherry and blackberry juices (pH < 3) (*7*, *36*), whereas it was lower in plum purée (pH > 3.5) (*35*) compared to the thermal stability of blueberry anthocyanins (pH 3.0) found in this study. It has also been suggested that sucrose has a protective effect on anthocyanins pigments at high temperatures (*46*), while reducing sugars enhance total anthocyanin degradation during heating, possibly due to the formation of furfural derivates (*47*).

Thermal Stability of Anthocyanins under Pressure. Thermal stability of anthocyanins in pasteurized blueberry juice under pressure was assessed at a range of selected conditions between 40 and 121 °C and 100 and 700 MPa at isothermal/isobaric conditions (please see **Table 4** for the specific $p-T$ combinations tested). Representative isothermal degradation kinetic curves of blueberry anthocyanins at 600 MPa and 40−121 °C are presented in **Figure 1B**. It is clear from parts **A** and **B** of **Figure 1** that the thermal stability of anthocyanins is reduced under pressure. The half-life for blueberry anthocyanins at ambient pressure and 90, 100, and 115 °C is approximately 115, 40, and 10 min (**Figure 1A**), whereas at 600 MPa it is reduced to 30, 20, and 5 min, respectively (**Figure 1B**). Intermediate pressures resulted in degradation rates only slightly higher than those obtained at ambient pressure and similar temperatures (kinetics not shown), indicating that the degradation of anthocyanins in blueberry juice is increased by pressure. This is not uncommon for chemical degradation reactions of food molecules (*20*, *48*) and in accordance with results of Corrales et al. (*12*), who reported a significantly higher reduction of cyanidin-3-glucosides in a model solution at 70 °C and 600 MPa compared to 70 °C at ambient pressure. High pressure may enhance the condensation reactions implicated in anthocyanin degradation in fruit juices, involving covalent association of anthocyanins with other flavonoids and organic acids present in fruit juices, leading to the formation of a new pyran ring by cycloaddition (*49*).

Application of 600 MPa at 40 °C resulted in 4% loss of blueberry anthocyanins after 15 min (**Figure 1B**). This moderate loss is in accordance with the reports that anthocyanins are generally stable during pressure treatments at moderate temperatures (*22*). For example, strawberry juice treated for 60 min at 65 °C and pressures up to 600 MPa did not show significant changes in visual color values (*50*). Insignificant degradation of anthocyanins was found after 15 min treatments at 500−600 and 800 MPa near room temperature in strawberry and blackberry purées (*11*) and in raspberries and strawberries, respectively (*18*, *51*).

Modeling Anthocyanin Degradation at Isothermal/Isobaric Conditions. Reports on thermal degradation of anthocyanins often describe their temperature dependence with the activation energy (E_a) of the Arrhenius model (eq 8) (*27*). Similarly, the pressure dependence of the degradation rate constant k can be expressed with the activation volume ($\Delta V^{\#}$), which can be obtained from the Eyring equation (eq 9) (*48*). Assuming first-order reactions, the rate constants for anthocyanin degradation at isothermal/isobaric conditions were calculated and the resulting activation volumes and activation energies are shown in **Tables 2** and **3**, respectively.

$\Delta V^{\#}$ was negative at all temperatures, indicating that the degradation reaction is generally accelerated by increasing pressure throughout the temperature range investigated. Surprisingly, the $\Delta V^{\#}$ slightly increased with increasing temperature. This reveals a relatively better pressure stability of anthocyanins in blueberry juice at high temperatures, which may be due to the predominance of thermal degradation at high temperatures.

At atmospheric pressure, the activation energy of blueberry anthocyanins was about 85 kJ mol^{-1}, which is lower than the reported E_a of 144 kJ mol^{-1} for anthocyanin degradation in

blueberry juice at pH 3.0 (*32*) but similar to the one found for anthocyanin degradation in elderberry concentrate (*44*) and in juices from blood orange (*33*, *34*) and purple or black carrot (*38*, *39*). The activation energy gradually decreased with increasing pressure (i.e., decrease in thermosensitivity of the degradation reaction) and was situated between about 70 and 52 kJ mol^{-1} in the pressure domain of 300−600 MPa (**Table 4**). **Figure 2** shows the Arrhenius plot for thermal degradation of anthocyanins in blueberry juice for the temperature range 40−121 °C at ambient pressure and at 600 MPa. In contrast to the degradation rate constants obtained at ambient pressure, it is obvious that the temperature dependence of the degradation rate constant at 600 MPa does not follow a true log−linear but a concave pattern (**Figure 2**). This possibly indicates that the degradation reaction follows different mechanisms at different temperatures (*37*) or that the up to 15 different blueberry anthocyanins (*40*) show different stabilities under pressure.

Close inspection of the isobaric/isothermal degradation kinetics of anthocyanins in blueberry juice indicated some deviations from simple first-order kinetics (**Figure 1**). This is likely due to the diverse anthocyanin composition where individual anthocyanins possess different thermal and possibly pressure stabilities (*3*, *31*). This leads to a distribution function of the overall anthocyanins content in blueberry juice and thus to a deviation from simple first-order degradation kinetics at high temperatures and pressures. The complete experimental data set was fitted to an *n*th-order reaction model (eq 6), with reaction orders varying from 1 to 1.8 in 0.05 increments. A uniform reaction order of 1.4 was found to give the minimum of the cumulative standard error function to describe best the time course of anthocyanin degradation at all $p-T$ conditions tested (data not shown). The lines in **Figure 1** interpolating the experimental data points show the fit using 1.4th-order kinetics. Upon fixing the reaction order, the specific degradation rate constant (k') remained as the only parameter in eq 5 and was estimated in a global approach using linear regression analysis (eqs 6 and 7). The resulting k' values of the different $p-T$ combinations investigated are shown in **Table 4**.

The kinetic data was also used to develop a mathematical model that describes the combined $p-T$ dependence of the specific degradation rate constant of blueberry anthocyanins. Empirical models such as eq 10 have been applied successfully to describe the combined effect of pressure and temperature on nutritional factors and degradation of food biopolymers (*20*, *48*). In this study, a third-order polynomial equation (eq 10) provided a good functional relationship of the obtained k' values with pressure and temperature. However, some variables of eq 10 did not significantly contribute to the models and eq 11 was used to eliminate model parameters until a minimum MSE was found. **Table 5** shows the parameter values derived from the model (eq 10), which were estimated by nonlinear regression fitting of eq 10 to the experimental data found for the $p-T$ combinations tested.

The parity plot of the natural logarithm of the experimental versus the predicted k' values (**Figure 3**) indicates no significant heteroskedasticity problems as the deviations from the bisector are small and thus the associated accuracy is high ($R^2 = 0.988$). Further assessment of the model yielded an accuracy factor (A_f, eq 12) value of 1.011, which corresponds to an error of the prediction of only 1.1%. Hence, the model is suitable to accurately describe the combined effect of pressure and temperature on the degradation rate of anthocyanins in blueberry juice.

Table 2. $\Delta V^{\#}$ and k_{ref} (at 0.1 MPa) Values for Pressure Degradation of Total Anthocyanins in Pasteurized Blueberry Juice at Different Temperatures

temperature (°C)	$\Delta V^{\#}$ (cm^3 mol^{-1})	k_{ref} (10^{-2} min^{-1})	R^2 [a]
60	−9.69 ± 2.16[b]	0.081	0.953
70	−8.26 ± 1.11	0.169	0.949
80	−6.12 ± 1.06	0.334	0.944
90	−5.18 ± 0.44	0.680	0.965
100	−3.45 ± 0.66	1.689	0.932
110	−4.66 ± 0.13	3.486	0.999
115	−3.10 ± 0.15	5.574	0.998

[a] Coefficient of determination. [b] Standard error of regression.

Table 3. E_a and k_{ref} (at 110 °C) Values for Heat Degradation of Total Anthocyanins in Blueberry Juice at Different Pressures

pressure (MPa)	E_a (kJ mol^{-1})	k_{ref} (10^{-2} min^{-1})	R^2 [a]
0.1	84.91 ± 1.70[b]	3.583	0.953
300	70.47 ± 15.96	4.703	0.949
400	57.29 ± 9.89	4.611	0.944
500	55.97 ± 18.77	6.453	0.965
600	51.81 ± 4.85	7.364	0.932

[a] Coefficient of determination. [b] Standard error of regression.

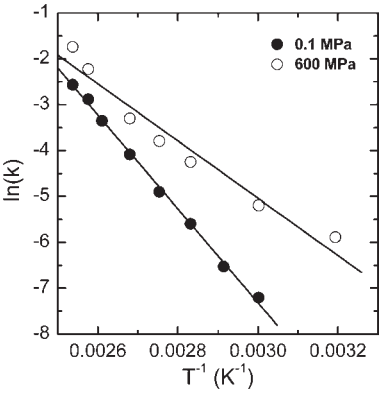

Figure 2. Arrhenius plot for thermal degradation of total anthocyanins in pasteurized blueberry juice at ambient pressure and at 600 MPa.

Table 4. Specific Rate Constants k' (10^{-2} min^{-1}) for Combined Pressure (p in MPa)−Temperature (T in °C) Degradation of Total Anthocyanins in Pasteurized Blueberry Juice Using a Reaction Order $n = 1.4$

	40 °C	60 °C	70 °C	80 °C	90 °C	100 °C	110 °C	115 °C	121 °C
0.1 MPa	0.006[a] ± 0.000[b]	0.070 ± 0.010	0.153 ± 0.015	0.409 ± 0.012	0.817 ± 0.048	2.103 ± 0.014	3.173 ± 0.254	8.076 ± 0.098	11.970 ± 0.325
100 MPa	nd[c]	nd	0.249 ± 0.015	nd	0.920 ± 0.155	nd	nd	nd	nd
200 MPa	nd	nd	nd	0.499 ± 0.064	nd	2.924 ± 0.268	nd	nd	nd
300 MPa	nd	nd	0.431 ± 0.142	nd	1.640 ± 0.151	nd	7.770 ± 0.604	nd	nd
400 MPa	nd	0.448 ± 0.081	nd	0.757 ± 0.071	1.070 ± 0.114	2.954 ± 0.255	nd	13.128 ± 0.233	nd
500 MPa	nd	nd	1.097 ± 0.135	nd	1.971 ± 0.184	nd	11.040 ± 2.461	nd	nd
600 MPa	0.284 ± 0.118	0.587 ± 0.078	nd	1.663 ± 0.140	2.954 ± 0.403	4.413 ± 0.253	nd	15.760 ± 0.412	29.251 ± 0.894
700 MPa	nd	nd	1.161 ± 0.079	nd	2.676 ± 0.211	nd	nd	nd	nd

[a] Taken from storage data. [b] Standard error of regression. [c] nd: not determined.

Table 5. Estimated Model Parameter Values for Degradation of Total Anthocyanins in Blueberry Juice Based on Equation 10 at a Reference Pressure of 600 MPa and a Reference Temperature of 40 °C

parameter	estimate
A	-5.699 ± 0.160[a]
B	$5.294 \times 10^{-3} \pm 4.175 \times 10^{-4}$
C	$3.099 \times 10^{-2} \pm 5.376 \times 10^{-3}$
D	$-1.018 \times 10^{-4} \pm 2.022 \times 10^{-5}$
E	
F	
G	
H	$3.733 \times 10^{-6} \pm 7.248 \times 10^{-7}$
I	
J	$6.741 \times 10^{-7} \pm 2.270 \times 10^{-7}$
R^{2} [b]	0.988
MSE	0.039
A_f	1.011

[a] Standard error of regression. [b] Coefficient of determination.

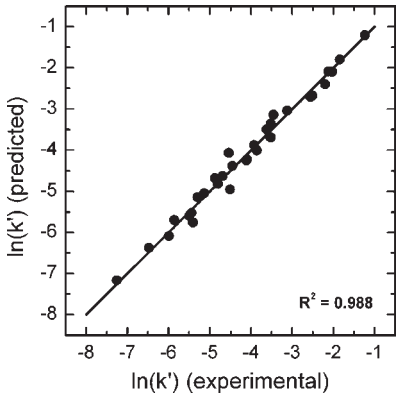

Figure 3. Correlation between the experimental *k*′ values of total anthocyanins in pasteurized blueberry juice determined at isothermal/isobaric conditions and the predicted *k*′ values using eq 10 with the parameters of **Table 4**.

Substituting k' in eq 6 by the $p-T$ relation of eq 10 allowed calculations of pressure−temperature isorate lines. Using the pressure as independent variable and setting the degradation ratio ($C \cdot C_0^{-1}$) and treatment time constant, the equation can be solved for the temperature. The $p-T$ diagram in **Figure 4** shows lines for 50% degradation (half-life) of blueberry anthocyanins after 5−300 min of isothermal/isobaric treatments. It is clear that temperature has a major effect on anthocyanin stability. The half-life of anthocyanins in blueberry juice is drastically reduced at high temperatures (>80 °C), and the degradation rate constants double every 8−11 °C at ambient pressure. The lines of constant degradation rates in **Figure 4** slightly bend to the left, indicating that there is a minor but noticeable decrease of the heat stability of blueberry anthocyanins at elevated pressures. This is similar to the heat stability of other small food molecules such as vitamins and betalains, which is reported to be slightly decreased under high pressures up to 850 MPa (*52*).

Anthocyanin Degradation during Storage. The effect of heat and combined $p-T$ treatments on the stability of anthocyanin in blueberry juice was also studied during storage at 4, 25, and 40 °C. **Figure 5** shows the change of relative anthocyanin concentration in blueberry juice treated at different conditions during storage at 25 and 40 °C. The degradation data for all three storage temperatures were fitted to a first and a 1.4th-order reaction model (eqs 3 and 6, respectively), and the obtained values are given in **Table 6**. The 1.4th-order reaction model was chosen on the basis of the best reaction order obtained in the thermal

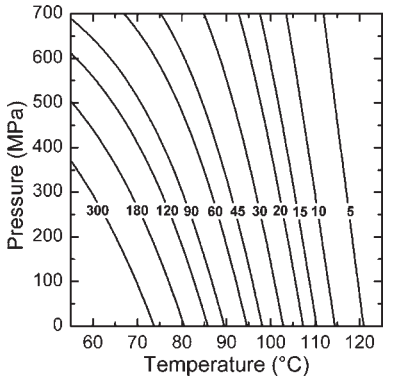

Figure 4. Pressure−temperature diagram for 50% degradation of total anthocyanins in pasteurized blueberry juice after 5−300 min isothermal/isobaric treatment.

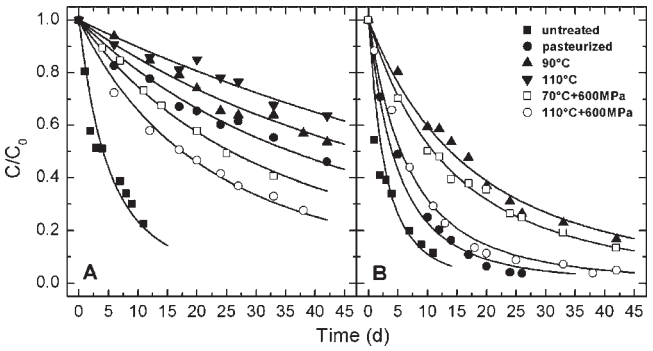

Figure 5. Degradation of total anthocyanins in differently treated blueberry juice (5 min at the conditions indicated) during storage at 25 °C (**A**) and 40 °C (**B**).

degradation studies and gave slightly better overall fit than the simple first-order model (see standard deviations in **Table 6**). The lines interpolating the kinetic data points in **Figure 5** show the fit of a 1.4th-order reaction scheme.

Total anthocyanin content in blueberry juice decreased through storage, at a rate strongly dependent on the storage temperature. Increasing storage temperatures resulted in a marked increase of the degradation rates (**Table 6**). The untreated juice showed by far the lowest anthocyanin stability, with almost 10-fold higher degradation rates at 4 and 25 °C compared to pasteurized juice (**Table 6**). The half-life of blueberry anthocyanins in pasteurized juice at 4, 25, and 40 °C was 184.3, 35.0, and only 5.1 days, respectively. Additional thermal processing of the pasteurized juice at 90 or 110 °C nearly always resulted in an increased (relative) stability of blueberry anthocyanins during storage compared to anthocyanin stability in pasteurized-only juices. The exception was when juices were stored at 4 °C, indicating that the degradation mechanism may be different at different storage temperatures. Combined high pressure thermal treatments did not show a clear trend to enhance or decrease anthocyanin stability compared to only pasteurized juice. Pressure processing of the blueberry juice at higher treatment temperatures tended to result in higher degradation rates at all storage temperatures (**Table 6**). For example, a 5 min treatment at 600 MPa and 70 °C resulted in anthocyanin half-lives of 203.4, 25.1, and 11.9 days, whereas a 5 min treatment at 600 MPa and 110 °C led to half-lives of 114.5, 18.3, and 5.8 days when stored at 4, 25, and 40 °C, respectively.

The total phenolic content of pasteurized blueberry juice was not significantly affected by pressure and heat treatment or

Table 6. Degradation Rate Constants (10^{-2} min^{-1}) of Total Anthocyanins in Untreated and Processed Blueberry Juice during Storage at 4, 25, and 40 °C, Respectively

processing conditions	temperature (°C)	k' [a] (d^{-1})	k [b] (d^{-1})	$t_{1/2}$(d)
untreated	4	4.634 ± 0.331[c]	4.280 ± 0.323	16.2
pasteurized[d]	4	0.386 ± 0.040	0.376 ± 0.039	184.3
90 °C[e]	4	0.281 ± 0.049	0.275 ± 0.047	251.7
110 °C[e]	4	0.441 ± 0.024	0.423 ± 0.021	163.8
600 MPa/70 °C[e]	4	0.345 ± 0.016	0.341 ± 0.016	203.4
600 MPa/90 °C[e]	4	0.288 ± 0.024	0.282 ± 0.023	245.7
600 MPa/110 °C[e]	4	0.631 ± 0.031	0.605 ± 0.030	114.5
untreated	25	19.611 ± 1.394	15.625 ± 1.510	4.4
pasteurized[d]	25	2.223 ± 0.084	1.981 ± 0.086	35.0
90 °C[e]	25	1.622 ± 0.071	1.484 ± 0.056	46.7
110 °C[e]	25	1.149 ± 0.054	1.078 ± 0.047	64.3
600 MPa/70 °C[e]	25	3.110 ± 0.077	2.764 ± 0.036	25.1
600 MPa/90 °C[e]	25	3.825 ± 0.059	3.110 ± 0.053	22.3
600 MPa/110 °C[e]	25	4.576 ± 0.128	3.780 ± 0.160	18.3
untreated	40	35.285 ± 1.217	27.175 ± 2.057	2.6
pasteurized[d]	40	20.060 ± 0.580	13.538 ± 0.372	5.1
90 °C[e]	40	5.749 ± 0.209	4.735 ± 0.225	14.6
110 °C[e]	40	6.486 ± 0.291	5.190 ± 0.292	13.4
600 MPa/70 °C[e]	40	7.271 ± 0.171	5.846 ± 0.227	11.9
600 MPa/90 °C[e]	40	14.497 ± 0.417	11.196 ± 0.244	6.2
600 MPa/110 °C[e]	40	18.045 ± 0.575	11.947 ± 0.447	5.8

[a] Specific degradation rate constant calculated with eq 6. [b] Degradation rate constant calculated with eq 3. [c] Standard error of regression. [d] 5 min pasteurization of the purée at 85 °C. [e] 5 min isothermal/isobaric treatment of pasteurized juice.

storage at 4, 25, or 40 °C for up to 27 days (data not shown). Furthermore, the pH of pasteurized blueberry juice decreased from 3.0 to 2.85 within approximately 6, 2, and 0.5 weeks of storage at 4, 25, and 40 °C, respectively, and was stable at this pH during further storage (data not shown). No noticeable difference from this pattern was found for juices treated at different $p-T$ combinations. Assuming that there is no microbial growth, such pH decrease of fruit juices during storage can be caused by pH decreasing decomposition products of anthocyanins such as phenolic acids (*53*). Furthermore, degradation of sugars to hydroxymethylfurfural and other Maillard products can lead to a pH decrease of the juice (*54*).

A relatively low stability of anthocyanins in different processed fruit juices during storage near room temperature has been reported (*27*). Anthocyanin half-lives at 25 °C ranged between 8 days in strawberry juice (*55*) to 47 days in grape juice (*38*). Tanchev (*32*) reported half-lives of 265, 87, 23, and 9 days for *Vaccinium myrtillus* anthocyanins in juice (pH 3) stored at 10, 20, 30, and 40 °C, respectively. This is slightly higher than the values found in this study (**Table 6**). Other works showed that the stability of individual anthocyanins can be different during storage (*56*, *57*). Thus, differences of the anthocyanin storage stability in different juices were attributed to their variation of anthocyanin composition (*7*). However, similar to thermal stability, there are many other environmental factors influencing the storage stability of anthocyanins including temperature, pH, oxygen, ascorbic acid, and anthocyanin degrading enzymes (*47*). The presence of native fruit enzymes such as PPO, POD, and β-glucosidases can cause a quick oxidation of anthocyanins (*3*−*5*) and most likely explains the poor storage stability of anthocyanins in the untreated juice of this study. The loss of anthocyanin pigments during storage in the absence of oxidative enzymes has been related to nonenzymatic oxidation as well as due to condensation of anthocyanin pigments with ascorbic acid or other flavonoids (*12*, *47*, *58*). Oxygen can either directly react with anthocyanins or oxidize other compounds that eventually react with anthocyanins to give colorless or brown products (*6*, *47*).

The quantity of ascorbic acid and its degradation byproducts have been reported to accelerate the degradation of anthocyanins during storage (*6*, *56*). Ascorbic acid is considerably reduced during processing at high temperatures. Thus, an increased storage stability of anthocyanins in heat-only treated juices might be due to the reduced concentration of oxidative or polymerizing agents. In addition, processing at high temperatures possibly has completely inactivated traces of undetected, temperature resistant oxidative enzymes still present in the pasteurized juice.

To date, literature data on the effect of high pressure processing on the storage stability of anthocyanins in the absence of enzymes is very limited. High pressure influences the equilibrium of chemical reactions and is likely to change physicochemical properties of blueberry juice under pressure. It is possible that the formation of *o*-quinones and other anthocyanin degrading radicals is enhanced at combined high pressure high temperature conditions causing an accelerated condensation and oxidation of anthocyanins during storage. On the other hand, ascorbic acid was reported to be more temperature labile under pressure (*52*). Thus, the $p-T$ treated blueberry juice is likely to contain less ascorbic acid than juice treated at the same temperature and ambient pressure condition. This may result in a slowed degradation of anthocyanins during storage (*15*). However, high pressure processing prevents evaporation of solubilized oxygen in the juice, which might occur during processing at high temperatures and ambient pressure. A higher oxygen level in the samples would thus lead to enhanced anthocyanin degradation during storage.

High pressure processing offers some unique opportunities to alter the rate of desired and undesired reactions in food. Synergistic effects of pressure and temperature are likely to enhance inactivation rates of vegetative microorganisms and some bacterial spores at a higher rate than the degradation of nutrients and color components (*20*, *21*). It is clear from **Table 6** that combined high $p-T$ treatments do not enhance the storage stability of anthocyanins in blueberry juice at ambient or higher storage temperature compared to heat-only treatments at 90 or 110 °C. On the other hand, microbial preservation of blueberry juice may be achieved by applying high pressure at much lower temperatures and shorter treatment times than commonly used in thermal preservation, which would possibly allow a better retention of anthocyanins and other volatile and bioactive and compounds. However, more studies on the combined effect of pressure and temperature on spoilage organisms and quality degrading enzymes are required to identify and design a safe high pressure process for blueberry juice preservation.

The kinetic data of this study extends the knowledge of the impact of combined high pressure−temperature treatments on the stability of anthocyanins and will possibly facilitate the inclusion of food quality aspects into the design of optimal conditions for high pressure sterilization of foods at elevated temperature. Analysis of individual anthocyanins as well as identification of anthocyanin degradation products with appropriate HPLC/LC-MS methods may highlight the advantages of high pressure processing over conventional heat processes.

ABBREVIATIONS USED

A, absorbance value; A, B, C, ..., J, polynomial coefficients; A_f, accuracy factor; C, anthocyanin concentration, mg L^{-1}; C_0, anthocyanin concentration at time = 0 mg L^{-1}; DF, dilution factor; E_a, activation energy, kJ mol^{-1}; k, degradation rate constant, min^{-1}, d^{-1}; k_{ref}, reference rate constant, min^{-1}; k',

specific rate constant, min^{-1}, d^{-1}; k'_{obs}, observed specific rate constant, min^{-1}; k'_{pred}, predicted specific rate constant, min^{-1}; MSE, mean square error; MW, molecular weight, g mol^{-1}; n, reaction order; N, number of degradation rate constants; p, pressure, MPa; p_{ref}, reference pressure, MPa; P, number of model parameters; POD, peroxidase; PPO, polyphenol oxidases; R, universal gas constant, 8.314 J mol^{-1} K^{-1}; T, temperature, °C, K; T_{ref}, reference temperature, K; t, time, min, s; $t_{1/2}$, half life, d; $\Delta V^{\#}$, activation volume, cm^3 mol^{-1}; ε, molecular extinction coefficient, L mol^{-1} cm^{-1}.

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