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Degradation kinetics of total anthocyanins and formation of polymeric color in blueberry hydrothermodynamic (HTD) processing

Alex Martynenko*, Yougui Chen

Department of Engineering, Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia, B2N 5E3, Canada

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ABSTRACT

Anthocyanin degradation and formation of polymeric color of blueberry puree in novel hydrothermodynamic (HTD) processing was studied. Anthocyanin degradation was non-significant in the range of temperatures from 2.5 to 80 °C, becoming significant above 80 °C ($p < 0.05$). Analysis of kinetic data indicated a first-order reaction for the degradation of blueberry anthocyanins with energy of activation 66.37 kJ/mol and half-lives ($t_{1/2}$) of 346.6, 187.3, 123.8, 80.6 and 38.7 min at temperatures of 70, 80, 87.5, 95 and 105 °C respectively. Polymeric color formation followed zero-order kinetics, progressively increasing with temperature. Polyphenol oxidase (PPO) and peroxidase (POD) were completely deactivated at 80 °C, which can be the reason of better shelf-life stability ($t_{1/2} = 533.2$ and 216.6 days at 4 and 20 °C, respectively). The beneficial aspect of technology on anthocyanin degradation and storage stability could be attributed to unique combination of temperature-pressure regimes of HTD processing.

1. Introduction

In recent decades the number of people, suffering from various chronic diseases including cardiovascular, diabetes, obesity and cancer, is rapidly increasing. As a better way of prevention and treatment of these diseases, WHO/FAO Expert Consultation (2002) has recommended changes in dietary practices towards natural foods. As a result, consumption of fresh and natural foods increased in recent years. Consumers usually link fresh and natural foods to specific nutritional attributes, such as vitamins, fiber and bioactive phytochemicals. Anthocyanins present a large group of red-blue polyphenolic pigments, which may help to prevent some human chronic diseases (Del Rio et al., 2013; Karlsen et al., 2007). Blueberries are a rich source of anthocyanins and have been recognized as one of the five major human health foods by Food and Agriculture Organization (FAO). There are more than 25 different anthocyanins in blueberries, which majorly are conjugated forms of malvidin, delphinidin, petunidin and cyanidin (Gao and Mazza, 1994). Many studies have reported that the blueberry anthocyanins not only play the crucial role in maintaining human health, but also treating and preventing diseases (Zafra-Stone et al., 2007; Norberto et al., 2013). Thus, anthocyanin content is usually used as an important quality indicator of blueberry products.

However, anthocyanins are very reactive and can be easily degraded to colorless or brown-color compounds (Kırca et al., 2007). The stability of anthocyanins in foods is influenced by a number of factors, including processing and storage conditions (temperature, oxygen, light), physical and chemical properties of foods (enzyme activity, pH and sugar content etc.) and presence of copigments and metallic ions (eg. hydrogen peroxide) (Jackman et al., 1987; Romero and Bakker, 2000; Kırca et al., 2007). Temperature is the most important factor that affects anthocyanin stability in both food processing and storage. Although most food can be consumed in natural form without any processing, the demand of global food market requires deep processing of food to extend the shelf-life. Heat processing is the most common and effective method to preserve food, but it may result in significant quality loss, mostly related to quick degradation of bioactive compounds (Sadilova et al., 2007; Patras et al., 2010). Elevated temperature can result in thermal degradation of monomeric anthocyanins and form polymers which is related to degradation of color and loss of nutritional values in food products. Many studies have suggested that increased pasteurization temperature results in higher degradation rate of anthocyanins in the range of temperatures from 70 to 121 °C (Brownmiller et al., 2008; Buckow et al., 2010).

* Corresponding author.
E-mail address: alex.martynenko@dal.ca (A. Martynenko).

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Abbreviations

HTD	hydrothermodynamic
HPP	high pressure processing
PEF	pulsed electric field
UV	ultraviolet
FAO	Food and Agriculture Organization
PPO	polyphenol oxidase
POD	peroxidase
k (s^{-1})	kinetic constant rate
$t_{1/2}$ (s)	half-life
E_a (kJ/mol)	activation energy
ΔG (kJ/mol)	free energy
ΔH (kJ/mol)	enthalpy
ΔS (J/mol K)	entropy
Q_{10}	temperature coefficient
R (8.314 J/mol K)	gas constant
h (6.6262×10^{-34} J s)	Planck's constant
k_B (1.3806×10^{-23} J/K)	Boltzmann's constant

In order to minimize thermal degradation of anthocyanins, a number of novel non-thermal technologies, such as high pressure processing (HPP), pulsed electric field (PEF), ultrasound and ultraviolet light (UV) irradiation have been proposed (Sen Gupta et al., 2005; Guerrero-Beltrán et al., 2009; Caminiti et al., 2011). It has been reported that these technologies preserve higher amount of anthocyanins, while are sufficient to kill spoilage microorganism. However, industrial applications of these novel technologies are limited due to high capital and operating costs. Recently discovered hydrotermodynamic (HTD) technology, based on phenomena of cavitation and turbulent friction in viscous liquids, combines benefits of low cost and high quality (Osipenko and Lesnikov, 2008). It was found that the combination of uniform temperature with high gradients of pressure create favorable conditions for a single-stage processing of raw blueberries (Martynenko et al., 2015). Preliminary research showed that HTD processing provided full pasteurization of blueberry puree at 95 °C with minimal anthocyanin loss (81% retention) (Satanina et al., 2014). Furthermore, Martynenko et al. (2015) recently reported that HTD processed blueberry product has shelf-life of more than 1.5 years for the retention of 50% of anthocyanins.

However, the mechanism behind these findings has not yet been studied. Additionally, optimization of the HTD processing conditions has neither been carried out. Absence of this fundamental knowledge limits the industrial applications of HTD technology. To fill this gap, the knowledge of anthocyanin degradation kinetics in HTD processing, including the reaction rate (k) as a function of temperature of processing, half-life ($t_{1/2}$) and activation energy (E_a), is required. Additionally, thermodynamic parameters including free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) could also provide valuable information concerning thermal stability of anthocyanins. Thermal degradation kinetics has been previously examined in blueberry juice with particular attention to different processing technologies (conventional heating and HPP) as well as storage studies (Kechinski et al., 2010; Buckow et al., 2010). The aim of this study was to investigate direct effect of HTD processing on thermal degradation of anthocyanins, as well as kinetics of anthocyanin degradation at temperatures above 70 °C.

Another aspect of anthocyanins degradation is related to enzymatic oxidation. Crushing of whole blueberries in HTD processing results in the release of native enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD) from the intact cell. If these enzymes are not completely deactivated, they could significantly decrease stability of anthocyanins during storage (Terefe et al., 2010). Traditional enzyme inactivation include heating and/or HPP in combination with thermal treatment (Buckow et al., 2010; Terefe et al., 2010). Considering importance of this effect on shelf-life stability, current study also examined the effect of HTD processing on PPO and POD activities.

2. Materials and methods

2.1. Materials

IQF Frozen blueberries, harvested in 2014, were supplied from PEI Berries Ltd (Montague, PEI, Canada) with initial moisture content 86.5 ± 0.7%, sugar content 7.8 ± 0.2 Brix and pH 3.31 ± 0.01. The frozen blueberries were stored at −20 °C until processing. All chemicals and reagents of analytical grade (Sigma–Aldrich, Oakville, Canada) were used without further purification.

2.2. HTD processing

Hydrothermodynamic (HTD) processing of blueberries was carried out using a pilot-scale HTD processor, consisting of a tank, centrifugal pump, temperature controller, temperature sensor, pressure gage and a Venturi cavitator (Martynenko et al., 2015). The principle of HTD processing is based on phenomena of high turbulence and cavitation in viscous liquid, resulting in simultaneous crushing, homogenization and pasteurization of whole berries in a closed system. The pump with head pressure 0.141 MPa and flow rate 0.5×10^{-3} m^3/s provided average velocity of food stream about 0.45 m/s, reaching 7.5 m/s in the active area of cavitation. Due to mechanical energy (pump action) input to the system, part of which gets utilized and released due to the cavitation in the closed system, temperature of product increased with the rate of 1.9–2.3 °C/min. As a result of thermal expansion in the closed system, the pressure inside of HTD processor increased from 0.02 to 0.3 MPa with temperature (Martynenko et al., 2015). The key processing variable under consideration in HTD processing is time, which affected both temperature and pressure. The temperature was measured with built-in thermocouples (Honeywell, USA) with accuracy of 0.1 °C and the pressure was measured with a pressure gage P16T2-4-100 (Indumart, Canada) with accuracy 1.6%.

Bulk frozen blueberries were thawed overnight (12 h) at room temperature prior to HTD processing. Partially unfrozen blueberries (5.5 kg) were quickly loaded in the tank by reaching the full capacity of HTD processor. The initial temperature of the blueberries before processing was approximately 0 °C. When the pump started moving the product, most of blueberries crushed during the first 2 min of processing to the median particle size about 0.8 mm. Further processing resulted in gradual decreasing of particle size to 0.27 mm, mostly due to the cavitation (Martynenko et al., 2015). Direct effect of HTD processing on anthocyanin thermal degradation was evaluated by periodical sampling of blueberry puree (~50 g) immediately when the temperature reached 2.5, 20, 35, 50, 65, 80, 95 and 105 °C. Samples were taken without interrupting the process through special sampling tap. Kinetics of anthocyanin degradation was studied by prolonged exposure of blueberry puree at different temperatures (70, 80, 87.5, 95, and 105°). To maintain constant temperature of the product, temperature controller turned the pump ON/OFF with the duty cycle 0.2–0.3 to balance heat generation and dissipation in the closed system. Eight to ten samples were taken at different holding times from 0 to 400 min. The temperature profiles and sampling points are shown in Fig. 1. All samples were collected in heat resistant centrifuge tubes and stored under −20 °C before chemical analysis.

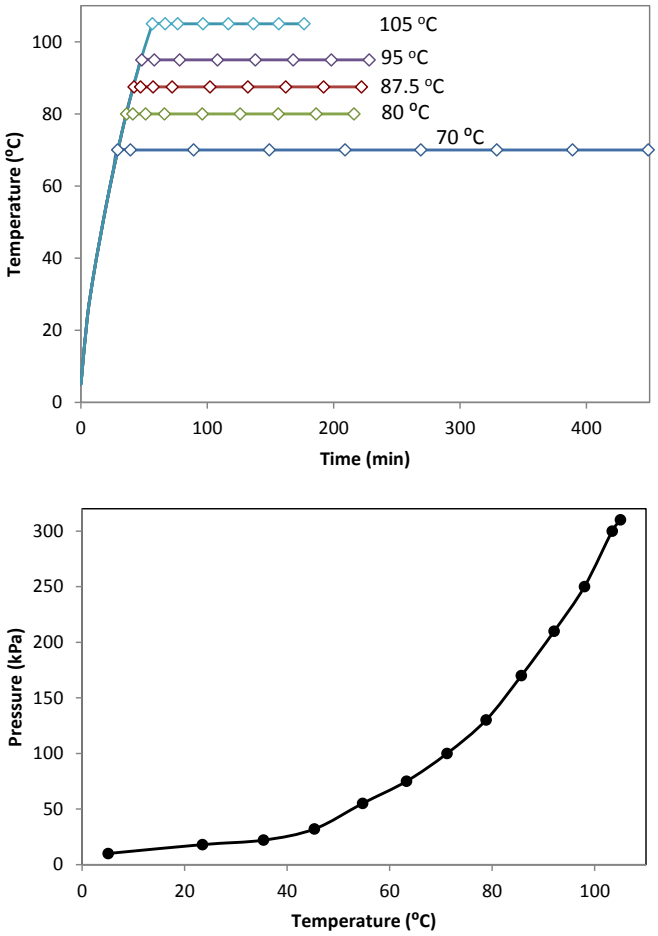

Fig. 1. Temperature (a) and pressure (b) profiles of blueberry HTD processing and sampling points (◊).

2.3. Extraction and determination of anthocyanins

Anthocyanin was solvent extracted according to Kalt's et al. (2008) protocol. HTD processed blueberry puree (15 g) was homogenized with 30 mL of cold extraction solvent which contained methanol, acetone and distilled water (2:2:1 v/v) with 0.1% formic acid for 3 min. The mixture was vacuum filtered through glass fiber #6 (Fisher Scientific, Whitby, Canada) and the extract was made up to a final volume of 50 mL with extraction solvent. The prepared extracts were stored under reduced light condition at −20 °C before analysis.

The monomeric anthocyanin content was determined according to pH differential spectrophotometric method (Giusti and Wrolstad, 2001) with some modifications in order to perform in 96-well microplates. Two buffer systems were used in the assay: potassium chloride buffer (0.025 M, pH = 1.0) and sodium acetate buffer (0.04 M; pH = 4.5). Aliquots of extract (10 μL) were transferred to a 96-well microplate and 290 μL of corresponding buffer (pH = 1 and pH = 4.5) and allowed to equilibrate for 30 min. The absorbance was measured at 520 nm and 700 nm, using a spectrophotometric microplate reader (BioTek, Winooski, USA).

The total anthocyanin content was calculated according to the following Formula (1):

$$Total\ Anthocyanin\ (mg\ C3G) = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot l} \quad (1)$$

where ε – molar extinction coefficient = 26,900 L/mol·cm for cyanidin-3-O-glucoside (C3G); l – path length of cuvette (1 cm); MW – molecular weight of C3G, DF – dilution factor, A – absorbance.

Absorbance was calculated by the following Equation (2):

$$A = (A_{pH\ 1.0} - A_{pH\ 4.5})_{525\ nm} - (A_{pH\ 1.0} - A_{pH\ 4.5})_{700\ nm} \quad (2)$$

2.4. Determination of polymeric color

Percent polymeric color was determined by measuring the absorbance after bleaching anthocyanin extract with 20% sodium metabisulfite to reveal only the color due to polymeric phenolic compounds (Giusti and Wrolstad, 2001). Simply, 10 μL was diluted with 190 μL 0.1% trifluoroacetic acid (TFA) in a 96-well microplate. For analysis, 25 μL of sodium metabisulfite was added to the diluted sample as bisulfite-bleached sample and 25 μL of 0.1% TFA was added as non-bleached, control sample and allowed to equilibrate for 30 min. Color density (CD) was calculated using the control sample according to the following Formula (3):

$$CD = [(A_{420\ nm} - A_{700\ nm}) + (A_{512\ nm} - A_{700\ nm})] \times DF \quad (3)$$

Polymeric color (PC) was determined using the bisulfite-bleached sample according to the same Formula (3). Percent polymeric color (PPC) was calculated using the following Formula (4):

$$PPC = PC/CD \times 100\% \quad (4)$$

2.5. Determination of PPO and POD activities

The analyses of PPO and POD enzyme activities were carried out as described by Terefe et al. (2010) with some modifications. The blueberry enzymes were extracted by 0.2 M sodium phosphate buffer (pH = 6.5) consisting of 4% (w/v) poly(vinylpyrrolidone) (PVP), 0.1% (v/v) triton X-100 and 1 M NaCl. Aliquot amount of blueberry puree (2 g) was homogenized with 2 mL extraction solvent for 3 min at 4 °C and centrifuged at 14,000× g for 30 min at 4 °C. The enzyme extracts were analyzed for PPO and POD activity on the day of preparation.

For PPO assay, aliquots of enzyme extracts (100 μL) were mixed with 3 mL of 0.07 M catechol in 0.05 M sodium phosphate buffer (pH = 6.5) solution. The absorbance was measured in a kinetic model at 420 nm at room temperature for 3 min, using a spectrophotometer (Spectronic 20, Bausch and Lomb, New Jersey, USA). For POD assay, 100 μL of enzyme extract was mixed with 2 mL of reaction mixture (25 mM guaiacol and 25 mM H_2O_2 in 0.05 M sodium phosphate buffer (pH = 6.5) solution. Both PPO and POD activities were expressed as the change of absorbance/min/g of sample fresh weight.

2.6. Evaluation of storage stability of anthocyanins

Blueberry samples processed at temperature of 95 °C without holding time were taken for storage stability study. Jars with HTD processed blueberry puree were divided into two groups and one group was stored at 4 and another group stored at 20–22 °C. Anthocyanin degradation was determined in triplicate on day 0, 8, 30, 48, 122, 189, 285, 376 and 546 days.

2.7. Kinetic data analysis

According to Patras et al. (2010), thermal degradation of anthocyanin is often expressed by a simple first-order reaction as following equation:

$$C_t = C_0 \exp(-kt) \quad (5)$$

where C_0 and C_t is anthocyanin concentration (mg C3G/g) at time 0 and t, k is the temperature-dependent rate constant (min^{-1}), and t is the heating time (min).

The half-life ($t_{1/2}$) of anthocyanins during heating was expressed as following equation:

$$t_{1/2} = -\frac{\ln(0.5)}{k} \quad (6)$$

To determine the effect of temperature on the kinetics of the anthocyanin degradation, the constants obtained from (5) were fitted to an Arrhenius Equation (7):

$$k = K_0 e^{-E_a/RT} \quad (7)$$

where, E_a is the Arrhenius activation energy (kJ/mol), K_0 is frequency factor (min^{-1}); R is the universal gas constant (8.314 J/mol K), and T is absolute temperature (K). Thus, the activation energy was calculated by plotting ln(k) against 1/T according to Equation (7).

Another parameter, namely temperature coefficient (Q_{10}), was also determined to characterize the effect of temperature on the rate of anthocyanin degradation and it was calculated by the following equation:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2-T_1)} \quad (8)$$

where $k_{1,2}$ are the rate constants (min^{-1}) at temperatures T_1 and T_2.

2.8. Thermodynamic analysis

Thermodynamic parameters of anthocyanin degradation, including activation enthalpy ($\Delta H^{\#}$), free energy of inactivation ($\Delta G^{\#}$), and activation entropy ($\Delta S^{\#}$), were determined by following equations established by Labuza (1980),

$$\Delta H^{\#} = E_a - RT \quad (9)$$

$$\Delta G^{\#} = -R \cdot T \cdot \ln\left(\frac{k \cdot h}{k_B \cdot T}\right) \quad (10)$$

$$\Delta S^{\#} = \frac{\Delta H^{\#} - \Delta G^{\#}}{T} \quad (11)$$

where h is he Planck's constant (6.6262×10^{-34} J s), and k_B is the Boltzmann's constant (1.3806×10^{-23} J/K).

2.9. Statistical analysis

All analyses were carried out at least in triplicate and results were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was done using Minitab 15.0 software (Minitab Inc., State College, PA, USA). Statistical significance was determined using least significant difference t-tests. Statistical significance was acceptable at $p < 0.05$.

3. Results and discussion

3.1. Thermal degradation of anthocyanins

The anthocyanin contents of blueberry puree during HTD processing at temperature from 5 °C to 105 °C are shown in Fig. 2A. The initial anthocyanin content of blueberry puree, measured after 2 min of processing at temperature about 5 °C, was 0.96 mg C3G/g. Further processing increased anthocyanin concentration to around 1.1 mg C3G/g at temperature of 20 °C, possibly due to the intensive release of anthocyanins from food matrix, skin and seeds. It is important to note that HTD processing didn't have significant effect of the anthocyanin content until temperature reached 65 °C, which is in agreement with Buckow's et al. (2010) finding that blueberry anthocyanins were very stable in juice up to 60 °C. Above this temperature the anthocyanin content rapidly decreased with further increase of temperature, reaching 0.80 mg C3G/g (27.2% loss) at temperature 105 °C.

Since polymerization is one of the most important reactions, which occurs during anthocyanin thermal degradation, percent polymeric color is a good indicator of anthocyanin degradation during both processing and storage (Türkyılmaz and Özkan, 2012). In our study polymeric color followed anthocyanin degradation, remaining at the same level below 65 °C (Fig. 2B). Further

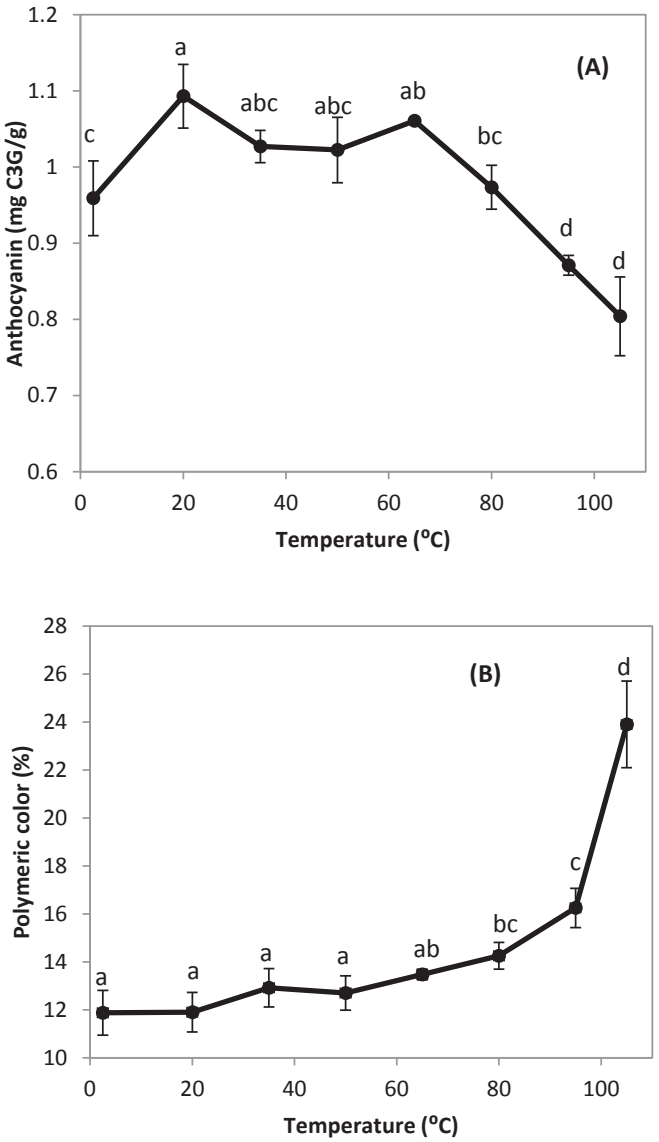

Fig. 2. Degradation of total anthocyanins (A) and polymeric color formation (B) in blueberry puree during HTD processing at temperature from 2.5 to 95 °C (average ± standard deviation, n = 3).

processing at elevated temperatures indicated remarkable formation of polymeric color. For example, temperatures of 80, 95 and 105 °C increased percent polymeric color to 14.3, 16.2 and 23.9%, respectively. The rate of polymeric color formation progressively increased with temperature, which might be explained by the formation of chalcone, an intermediate product of anthocyanin degradation (Patras et al., 2010). The chalcone is unstable and could be quickly degraded to brown color products, resulting in significant increase of polymeric color.

3.2. Kinetics of anthocyanin thermal degradation

Kinetics of anthocyanin thermal degradation was studied by isothermal exposure of blueberry puree at different temperatures from 70 to 105 °C and plotted as a function of holding time (Fig. 3). Strong first-order degradation kinetics observed at all temperatures ($R^2 > 0.88$) was in agreement with previous studies of anthocyanin thermal degradation in blueberry juice (Buckow et al., 2010; Kechinski et al., 2010). Kinetic parameters, including kinetic rate constant (k) and half-life ($t_{1/2}$), were calculated from Fig. 3 and presented in Table 1. As expected, the k values increased with temperature, indicating that greater degradation occurs at higher processing temperatures. The calculated $t_{1/2}$ values were 346.6, 187.3, 123.8, 80.6, and 38.7 min at 70, 80, 87.5, 95 and 105 °C respectively. These values were slightly lower than the results of blueberry juice mild heating study (Kechinski et al., 2010), who reported half-life of anthocyanins as 516 and 306.6 min at 70 and 80 °C. Minor decrease of anthocyanin stability in HTD processing at temperatures below 80 °C could be explained by combined effect of temperature and processing time. However, at higher temperatures (95 and 105 °C), the $t_{1/2}$ values were better than reported by Buckow et al. (2010), who found 115 and 40 min at 90 and 100 °C respectively. This comparison indicated that HTD processing at temperatures above 90 °C provides less anthocyanin degradation than conventional pasteurization.

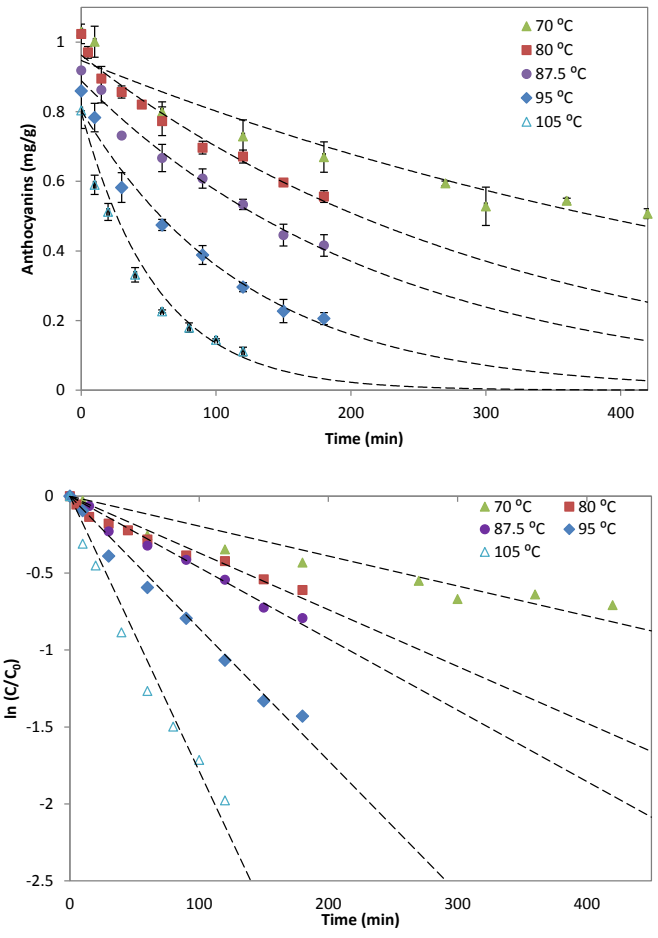

Fig. 3. Thermal degradation kinetics of total anthocyanins in blueberry puree in HTD processing in the range of temperatures from 70 to 105 °C (Note: broken lines represent the behavior predicted by the pseudo first-order kinetic model).

Activation energy (E_a) is normally used to describe the energy required to reach the transition state of a reaction (Lodish et al., 2000). The activation energy is usually evaluated from experimental data by using Arrhenius model. Fig. 4 presents the Arrhenius plot developed from the kinetic constant rates of anthocyanin, obtained in this study. It follows that overall experimental data in the range of temperatures from 70 to 105 °C fit very well with the Arrhenius model ($R^2 = 0.99$). The calculated value of E_a was 66.37 kJ/mol, which is much lower than reported 80–144 kJ/mol for anthocyanin degradation in blueberry juice using conventional pasteurization (Tančev, 1974; Kechinski et al., 2010; Buckow et al., 2010). The lower E_a indicated that blueberry anthocyanin in HTD processing is less susceptible to degradation by exposure to increased temperatures as compared to conventional thermal processing. This could be the synergetic effect of cavitation, pressure and heat in novel HTD processing, which is in agreement with Buckow's et al. (2010) study that activation energy was rapidly decreased with elevated pressure due to the decreased thermosensitivity of anthocyanin degradation. Although the experimental k values were generally fit well on the Arrhenius plot, it was noted that k values at extremely high temperature (105 °C) was slightly above the regression line. Thus, E_a was re-calculated for smaller temperature ranges to find out whether the activation energy is affected by temperature. Interestingly, it was found that the E_a was much higher in the temperature range from 95 to 105 °C (84.84 kJ/mol) as compared to lower temperatures (58.5–63.1 kJ/mol) (Table 2). This indicated that anthocyanins became more sensitive to thermal degradation at temperatures from 95 to 105 °C. This result for HTD processing is in agreement with conclusions of other researchers that E_a value is increasing with processing temperature (Harbourne et al., 2008). In addition to E_a, temperature coefficient (Q_{10}) provided similar information regarding to the effect of temperature on anthocyanin degradation. Q_{10} values were calculated as 1.74 to 2.08 (Table 2) in the different temperature ranges, which is in agreement with other studies (Kechinski et al., 2010).

Estimation of thermodynamic parameters could provide additional and useful information for thermal degradation kinetics. The activation enthalpy (ΔH), Gibbs free energy (ΔG) and activation entropy (ΔS) at different temperatures in HTD processing are presented in Table 3. $\Delta H^{\#}$ represents the minimum energy required for the reactant to make the reaction occurred and is related to the strength of the chemical bonds, which are broken and made during the reaction (Vikram et al., 2005). ΔH of anthocyanin thermal degradation in HTD determined in this study was similar at different temperatures, ranging from 63.20 to 63.49 kJ/mol. The positive value of $\Delta H^{\#}$ indicated that the reaction of anthocyanin

Table 1
Effect of temperature on the *k* and $t_{1/2}$ values of anthocyanin degradation in the HTD processed blueberry puree.

Temperature (°C)	*k* (min^{-1})	$t_{1/2}$ (min)	r^2	E_a[a] (kJ/mol)
70	−0.002	346.6	0.8815	66.37
80	−0.0037	187.3	0.9329	
87.5	−0.0056	123.8	0.9786	
95	−0.0086	80.6	0.9449	
105	−0.0179	38.7	0.9635	

[a] Activation energy calculated from Arrhenius plot (Fig. 3).

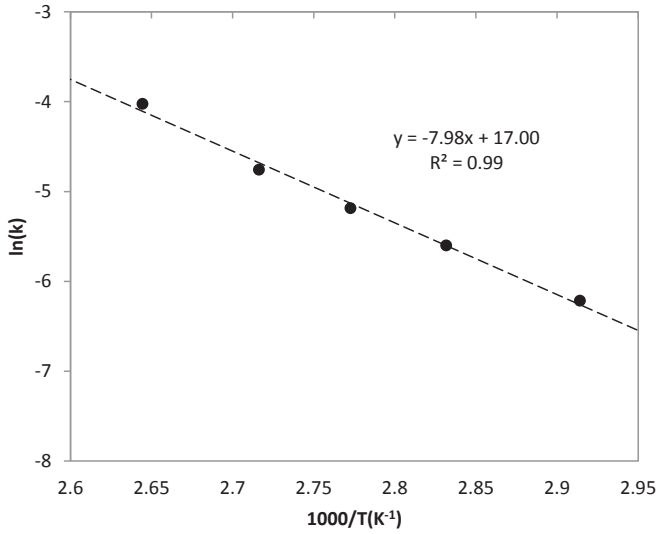

Fig. 4. Arrhenius plot for anthocyanin degradation of the blueberry puree in HTD processing in the range of temperatures from 70 to 105 °C (Note: broken lines represent the behavior predicted by the zero-order kinetic model).

Table 2
Effect of temperature on Q_{10} and E_a values of blueberry anthocyanin degradation in HTD processing.

Temperature (°C)	Q_{10}	E_a (kJ/mol)
70 to 80	1.85	61.98
80 to 87.5	1.74	58.51
87.5 to 95	1.77	63.14
95 to 105	2.08	84.84

degradation is an endothermic reaction and it proves our previous results that the degradation rate increased with temperature (Table 1). The Gibbs free energy ΔG is defined as the difference between energies of reactants and activated state and is usually served as a measure of process spontaneity (Mercali et al., 2013). Positive ΔG values (102.17–106.00 kJ/mol) indicated that anthocyanin thermal degradation is non-spontaneous reaction. Both ΔH and ΔG values obtained in current study are similar to the values reported by Kechinski et al. (2010) for blueberry juice as 77.8 and 91.3 kJ/mol for ΔH and ΔG, respectively. ΔS implies the change of disorder of molecules in the reaction system and it is usually related to the number of molecules with appropriate energy that can actually react (Vikram et al., 2005). ΔS values determined in this study were all negative, varying from −114.31 to −112.72 J/mol K, indicating that the transition state has less structural freedom than the reactants. Additionally, the ΔS values were relatively higher than that reported by Kechinski et al. (2010) of −43.07 kJ/mol. This implies that more energy is required to form an activated complex (Al-Zubaidy and Khalil, 2007) and is definitely beneficial feature of HTD processing.

Table 3
Thermodynamic parameters obtained for blueberry anthocyanin degradation during HTD processing.

Temperature (°C)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol K)
70	63.49	102.17	−112.72
80	63.41	103.43	−113.32
87.5	63.34	104.44	−113.95
95	63.28	105.37	−114.31
105	63.20	106.00	−113.20

3.3. Kinetics of polymeric color formation

Kinetics of polymeric color formation during HTD processing of blueberries was studied by plotting PPC as a function of time at different temperatures (Fig. 5). It was observed that kinetics followed zero-order reaction with high determination coefficient ($R^2 = 0.92–0.99$). Similar zero-order relationship of polymeric color formation was reported in black carrot juice storage study (Türkyılmaz and Özkan, 2012). The rate constants (k) for formation of polymeric color were 0.0262, 0.0369, 0.062, 0.1645, and 0.2595%/min at 70, 80, 87.5, 95 and 105 °C, respectively (Table 4). Similar with anthocyanin degradation, the k values of polymeric color formation increased significantly with temperature.

Calculated temperature coefficients (Q_{10}) for polymeric color formation at different temperature ranges are presented in Table 4. Similar with anthocyanin degradation, Q_{10} values for PPC significantly increased from 1.41 to 3.67 with temperature elevation in the range from 70 to 80 to 87.5–95 °C. This fact could be explained by the accelerated formation of chalcone at high temperatures as mentioned preciously. In contrast, Q_{10} decreased rapidly with further increase of temperature in the range 95–105 °C. This result may be due to decreased phenolic content in blueberries at temperatures above 95 °C. The polymerization reaction of anthocyanin mostly occurs by bounding the monomeric anthocyanins with other phenolic compounds, such as phenolic acid and condensed tannins (Türkyılmaz and Özkan, 2012), thus the observed behavior could be explained by substrate-limited reaction.

Fig. 6 shows strong negative correlation between the anthocyanin content and PPC in HTD processing of blueberries, which could be expressed by exponential relationship:

$$PPC = 10 + 79.95 \cdot e^{-3.405 \cdot C}$$

where C is anthocyanin content, mg C3G/g.

It indicates that degradation of anthocyanins is always accompanied by the formation of polymeric color, which is in agreement with Türkyılmaz and Özkan's (2012) observation in black carrot juice. Our results suggest that polymeric color can be used as an excellent indicator for anthocyanin degradation in blueberry HTD processing.

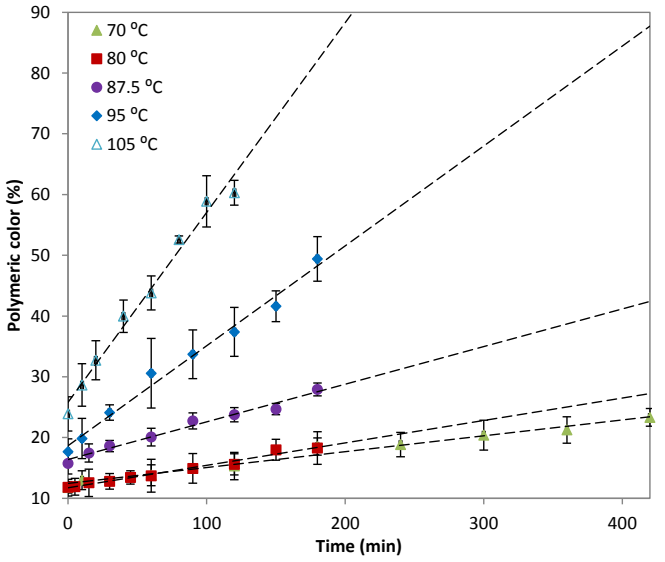

Fig. 5. Formation of polymeric color of the blueberry puree in HTD processing in the range of temperatures from 70 to 105 °C (Note: broken lines represent the behavior predicted by the zero-order kinetic model).

Table 4
Linear approximation of relationship of percent polymeric color and holding time of the blueberry puree in HTD processing in the range of temperatures from 70 to 105 °C.

Temperature (°C)	k (%/min)	R^2		Q_{10}
70	0.0262	0.9777		
80	0.0369	0.9797	**70–80 °C**	1.41
87.5	0.062	0.9803	**80–87.5 °C**	2.00
95	0.1645	0.9871	**87.5–95 °C**	3.67
105	0.2595	0.9205	**95–105 °C**	1.58

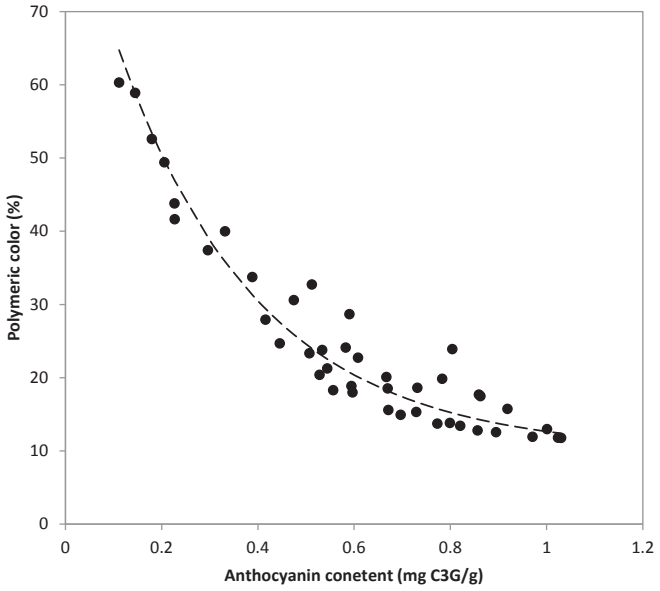

Fig. 6. Correlation between anthocyanin content and polymeric color during blueberry HTD processing (Note: broken lines represent the behavior predicted by the pseudo first-order kinetic model).

3.4. Thermal inactivation of polyphenol oxidase (PPO) and peroxidase (POD) and storage stability of anthocyanins in HTD processed puree

Polyphenol oxidase (PPO) and peroxidase (POD) are the two major enzymes naturally present in blueberries, directly related to anthocyanin degradation during processing and storage. It has been found that incomplete deactivation of PPO and POD would significantly affect storage stability of anthocyanins and other polyphenols in fruit products (López-Serrano and Ros Barceló, 2002; Chisari et al., 2007). The effect of temperature on PPO and POD during HTD processing was shown in Fig. 7. Significant increase of PPO activity was observed at the beginning of HTD processing with the highest activity at 20 °C. This can be explained by quick crashing of cellular structure and release of membrane bound PPO into solution. However, the PPO activity decreased significantly with the increase of temperature above 35 °C and was completely inactivated when temperature reached 80 °C. Previous research revealed high thermostability of PPO in soft fruits and berries, such as blueberry and strawberries (Terefe et al., 2010). Usually, inactivation of PPO requires 3–4 min blanching at temperatures above 85 °C (Rossi et al., 2003; Gao et al., 2012). The current results clearly demonstrated the benefits of HTD processing for the immediate PPO inactivation at temperature 80 °C. This could be explained by the combined effect of temperature and cavitation. Cavitation creates significant local pressure gradients in the food stream, which could affect protein structure and activity (Martynenko et al., 2015). Extremely high energy gradients and turbulence could also have significant effect on inactivating PPO. Considering importance of this finding for industrial processing, further studies on the mechanism of inactivation of PPO in HTD processing are needed.

The effect of temperature on POD during HTD processing was similar. The POD activity slightly increased at beginning of HTD processing (T < 35 °C), but then inactivated faster than PPO. Fig. 7B shows that POD was completely inactivated at 65 °C. It is common that POD could be easily inactivated at temperature 70 °C for 5 min (Terefe et al., 2010; Chisari et al., 2007).

To further identify the shelf-life stability of anthocyanins in HTD processed blueberry puree, shelf-life study of anthocyanin degradation during 18 months of storage at temperatures 4 °C and 20 °C was carried out. Kinetics of anthocyanin degradation during storage demonstrated first-order behavior with $R^2 = 0.95–0.98$ (Fig. 8). The activation energy was calculated as 24.40–29.98 kJ/mol, which is much lower than previously reported data on fruit juice products, where E_a value was ranging from 62.1 to 84 kJ/mol (Kırca and Cemeroğlu, 2003; Kırca et al., 2007; Turker et al., 2004). The half-lives of the HTD processed blueberry puree were calculated as 533.2 and 216.6 days at 4 and 20 °C, respectively. These values are also much higher than that of the conventional pasteurized juice as 184.3 and 35 days for 4 and 25 °C respectively. The superior storage stability of HTD processed blueberry puree could be explained by complete inactivation of enzymes.

Overall, the significant effectiveness of HTD processing on inactivating PPO and POD and extending shelf stability of the products demonstrated the potential of HTD processing for producing premium quality and functional food with long shelf-life.

4. Conclusions

The present study evaluated the effect of HTD processing parameters, namely temperature and exposure time, on the thermal

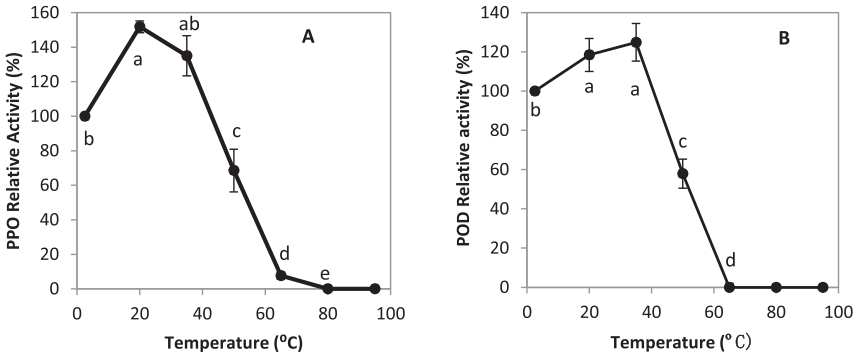

Fig. 7. Thermal stability of blueberry PPO (A) and POD (B) in HTD processed blueberry puree. The relative activity of PPO and POD is defined as the ratio of the heat treated sample to untreated sample (average ± standard deviation, n = 3).

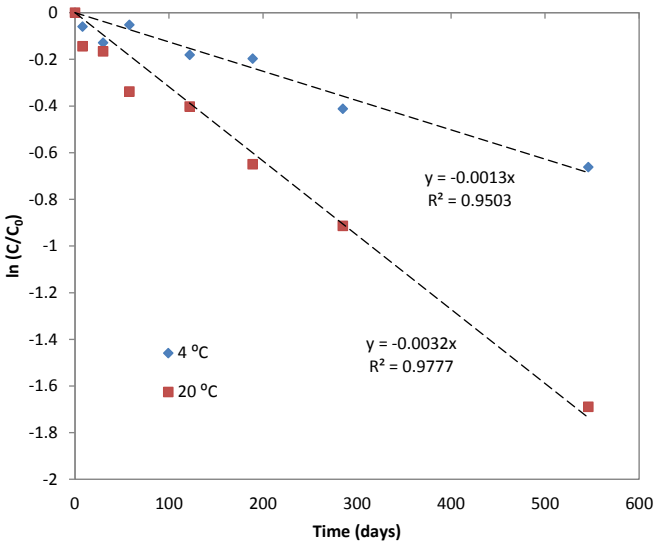

Fig. 8. Thermal degradation kinetics of total anthocyanins in HTD processed blueberry puree at storage temperatures (Note: broken lines represent the behavior predicted by the zero-order kinetic model).

degradation of anthocyanin, formation of polymeric color and enzymes inactivation. Anthocyanins content was stable in the range of temperatures up to 80 °C. At this temperature PPO and POD were completely inactivated. Kinetics of thermal degradation of anthocyanins in HTD processing followed first-order reaction with activation energy 66.37 kJ/mol. Our results showed that anthocyanins in HTD processing were less susceptible to temperature than in conventional thermal processing. Also, HTD processed blueberry puree had much longer shelf-life of about 1.5 years regarding to anthocyanin retention.

The results demonstrated that HTD processing could offer some unique opportunities to produce functional foods, rich in anthocyanins and other bioactive phenolics. The kinetic data extend our knowledge about effects of HTD processing on the stability of anthocyanins, which is important for further industrial optimization.

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