

Degradation kinetics of anthocyanins in blackberry pulp during ohmic and conventional heating

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Article history

Received: 6 October, 2017

Received in revised form:

23 April, 2018

Accepted: 30 May, 2018

Keywords

Thermal treatment

Electric field

First order kinetic model

Blackberry pulp

Abstract

Anthocyanin degradation kinetics in blackberry pulp during ohmic and conventional heating was evaluated at different temperature conditions (70, 75, 80, 90°C). Ohmic heating experiments were performed at 23 V and 125 V (60 Hz). The anthocyanin degradation rates were similar for both heating technologies for low voltage (23 V). Nevertheless, when high voltages were applied, anthocyanin degradation increased. This result can be attributed to the electrochemical reactions that might contribute to greater anthocyanins degradation during ohmic heating. The first order model provided a good description of the degradation kinetics for both heating technologies, and the k values ranged from 0.0155 to 0.0051 min⁻¹. The activation energies were 67 and 56 kJ mol⁻¹ for ohmic and conventional heating, respectively. ΔH^\ddagger , ΔS^\ddagger and ΔG^\ddagger values were similar for all conditions, varying between 53.6 to 64 kJ mol⁻¹, 102.3 to 105.5 kJ mol⁻¹ and -114 to 144 J mol⁻¹ K⁻¹, respectively.

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Introduction

The growing market demand on bioactive compound rich products highlights the importance of preserving the stability of these compounds during processing. Blackberries are a good source of anthocyanins (Sariburun *et al.*, 2010; Haminiuk *et al.*, 2012; Kaume *et al.*, 2012), and are frequently submitted to thermal processes to ensure food safety. Several studies throughout the years have provided evidence that an anthocyanin-rich diet is associated with probable reduced risks of cardiovascular disease, cancer, inflammation, obesity, diabetes and other diseases (Kaume *et al.*, 2012; Norberto *et al.*, 2013; Peluso *et al.*, 2015). Anthocyanins are polyphenolic pigments; they are glycosylated polyhydroxyl or polymethoxyl derivatives of the 2-phenylbenzopyrylium (flavylium) cation. These compounds are potent antioxidants, having high free-radical scavenging and metal chelating activities (Bravo, 1998; Francis and Markakis, 1989).

Blackberries are often consumed raw. However, the fragility and high post-harvest respiration rate of

these fruits significantly contributes to their nutritional and microbiological deterioration, and also to their decay and firmness loss, limiting the shelf-life and diminishing quality and health benefits (Bower, 2007; Sariburun *et al.*, 2010). To fulfil consumers' demands and market needs, it is therefore necessary to store products for longer periods, especially in between crops. Therefore, most fresh blackberries, as well as other fruits, are processed into jams, jellies and juices, or converted into frozen, dried and canned products (Rickman *et al.*, 2007). Numerous researches have showed the effects of processing on the bioactive compounds of blackberries (Wu *et al.*, 2010; Howard *et al.*, 2012; Jiménez *et al.*, 2012; de Souza *et al.*, 2015). Since thermal processing is one of the most widely used methods for food conservation, and that heat is an important factor affecting the stability of anthocyanins, the kinetic parameters of the heat degradation of anthocyanins (activation energy and reaction rate constant) have been studied for a broad variety of products (Loypimai *et al.*, 2015; Martynenko and Chen, 2016; Ziabakhsh Deylami *et al.*, 2016).

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The kinetic degradation of nutrients during thermal processing and storage of foods has been conventionally described in terms of zero, first or higher orders (Corradini and Peleg, 2006). Anthocyanins degradation during thermal processing has been particularly fitted to first-order kinetics (Wang and Xu, 2007; Loypimai *et al.*, 2015; Martynenko and Chen, 2016; Turturicǎ *et al.*, 2016). However, there is enough experimental evidence that the isothermal degradation curves of many compounds and the survival curves of many bacterial cells are not log linear as the first-order kinetics model requires (Aragao *et al.*, 2007). The Weibull model has an interesting potential for describing microbial and enzymatic inactivation and chemical degradation kinetics (Cunha *et al.*, 1998). Ziabakhsh Deylami *et al.* (2016) evaluated PPO inactivation, anthocyanin loss and colour changes of mangosteen pericarp during blanching under a temperature range of 60 to 100°C. The researchers reported that anthocyanins degradation was better described by the Weibull kinetic model.

The Arrhenius equation is an empirical collision model often used to describe the temperature dependence of the reaction rate. An alternative approach to the Arrhenius equation is the Eyring equation, a theoretical construct, based on transition state model, used in chemical kinetics to describe the variance of the rate of a chemical reaction with temperature. The Ball model is another possibility frequently used in food processing for microbial inactivation.

An alternative to conventional thermal process is ohmic heating. The main advantage of this heating method is the rapid and uniform heating process, which results in minimal losses of the structural, nutritional and sensorial properties of the food. In ohmic heating the electrical resistance of the food itself generates heat as current is passed through it. Most foodstuffs are naturally resistant to the passage of electrical current and able to convert electrical energy into thermal energy. The temperature increases without the need of heat transfer through solid-liquid interfaces (Sastry, 2008). This process has many potential applications, including thawing, blanching, evaporation, dehydration, fermentation and pasteurisation, among others (FDA, 2010). Therefore, it is important to evaluate the effect of ohmic heating on anthocyanin degradation and to compare this effect with that exerted by conventional heating method. Studies have evaluated the effect of ohmic heating on vitamin C degradation, and determined the kinetic parameters for this process (Assiry *et al.*, 2003; Castro *et al.*, 2004; Louarme

and Billaud, 2012). However, to the best of our knowledge, the published data on the kinetics of anthocyanin degradation during ohmic heating are still lacking (Mercali *et al.*, 2013; Sarkis *et al.*, 2013a; Mercali *et al.*, 2015).

To preserve anthocyanins, it is important to study and model their degradation, in order to predict their concentrations according to different processes, times and temperatures used. An accurate knowledge of the kinetic parameters allows for better understanding and prediction of the changes undergone by anthocyanins during processing. Therefore, the objective of the present work was to obtain an adequate kinetic model for anthocyanin degradation in blackberry pulp during the isothermal processing at different temperatures (70-90°C) using two different thermal treatments: ohmic and conventional heating. Moreover, the work also aimed to evaluate the non-thermal effects of the electric field on blackberry anthocyanins and collaborate for the correct understanding of the non-thermal effects associated with the passage of electricity through the foodstuff on degradation kinetics. The present work would have significant economic consequences to the industry since they enable modelling and optimisation to produce safe products with higher nutritional and sensorial characteristics.

Materials and methods

Blackberry pulp

Blackberry (*Rubus* sp.) pulp was supplied by Mais Fruta Company (Antônio Prado, RS, Brazil). The fruits in the pulp belonged to two different cultivars; “Tupi” and “Guarani”. The pulp production included washing, sorting, extraction, cooling, packaging and freezing. The product was not pasteurised during processing. The samples were received frozen in packs of 100 g, and was stored at -18°C for later analyses. On the day of the experiment, the sample was thawed at room temperature (approximately 20°C).

Ohmic heating

The experimental setup for ohmic heating comprised a power supply, a variable transformer from 0 to 240 V (Sociedade Técnica Paulista LTDA, model Varivolt, Brazil), a stabiliser (Forceline, model EV 1000 T/2-2, Brazil), a data acquisition system, a computer and an ohmic cell. The experimental apparatus is schematically shown in Sarkis *et al.* (2013c). The voltage across and the current through the heated sample were measured using voltage and current transducers. The temperature was monitored

by two stainless steel temperature sensors (Novus, model pt-100, Porto Alegre, RS, Brazil) coated with a nickel-phosphorous alloy. These variables were recorded at constant time intervals by a data logger (Novus, model Field logger, Porto Alegre, Brazil) linked to the computer. Frequency was kept constant at 60 Hz. The Pyrex glass ohmic cell had a capacity of 400 mL, and was equipped with a water jacket. The lid of the vessel contained four ports for temperature sensors and two ports for the electrodes. The electrodes were made of platinum, with cross-sectional areas of 7.0 cm². The electrode gap was 5.6 cm. The cell was placed on a magnetic stirrer plate (Instrulab, Model ARE, Porto Alegre, RS, Brazil) to allow for agitation of the product during heating.

The kinetics experiments were conducted at 70, 75, 80 and 90°C. The initial temperature of the samples before heating was approximately 20 ± 1°C. The samples were heated using water in a thermostatic bath (Lauda, model TYP T, Germany). When the sample reached the desired temperature, the heating water bath was turned off, and the voltage was turned on, and kept at 23 ± 2 V (frequency of 60 Hz) for 90 min. The hot water was chosen to heat up the sample until the desired holding temperature in order to equalise the temperature profiles for both heating methods (ohmic and conventional). This procedure eliminated the effect of the heating as a variable during the experiments and allowed for a fair comparison between methods (conventional and ohmic) during the isothermal step.

Samples were withdrawn at various heating times (0, 15, 30, 45, 60, 75 and 90 min), and monomeric anthocyanin concentrations were determined for each sample. The anthocyanin content after the sample reached the desired holding temperature (time zero) was considered the initial anthocyanin content.

A similar experiment was performed, using a voltage of 125 ± 5 V and keeping the sample at 80°C. In this case, a cooling bath at 4°C was used to maintain the temperature constant.

Conventional heating

The conventional heating processing was carried out in a 200 mL Pyrex glass vessel equipped with a water jacket. A thermostatic water bath (Lauda, model T, Germany) was used to heat the samples. The vessel was kept on a magnetic stirrer plate (Instrulab, Model ARE, Porto Alegre, RS, Brazil) to promote agitation of the blackberry pulp during heating. During the experiments, the temperature was monitored using type T thermocouples and a data acquisition system (Novus, model Field logger, Porto Alegre, RS, Brazil), which was linked to a

computer. The conventional kinetics experiments were conducted at the same temperature and for the same period as the ohmic heating experiments.

Quantification of anthocyanins

During the experiments, 2 mL aliquots of blackberry pulp were collected in Eppendorf tubes. The samples were taken (1) before the heating started (fresh), (2) when the desired temperature was reached, and (3) at the aforementioned intervals. The samples were cooled immediately after they were collected and centrifuged (4°C, 4757 g, 30 min). The blackberry juice, which was the supernatant, was used for the quantification of anthocyanins.

The pH differential method described by Giusti and Wrolstad (2001) was used for the total monomeric anthocyanin content determination. The blackberry juice was dissolved in a potassium chloride buffer of pH 1.0 (KCl, 0.025 M) and, separately, in a sodium acetate buffer of pH 4.0 (CH₃CO₂Na·3H₂O, 0.4 M) with a pre-determined dilution factor (DF). The absorbance of the samples were measured at 510 nm (A₅₁₀) and 700 nm (A₇₀₀) against a blank cell containing distilled water. Following readings, the absorbance of the diluted sample (A) was calculated using Equation 1 as follows:

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5} \quad \text{Equation 1}$$

The monomeric anthocyanin pigment concentration in the sample in mg L⁻¹ ([Acy]) was calculated using Equation 2 as follows:

$$[\text{Acy}] = (A \cdot \text{MW} \cdot \text{DF} \cdot 1000) / (\epsilon \cdot l) \quad \text{Equation 2}$$

In this equation, cyanidin-3-glucoside molecular weight (MW = 449.2) and molar absorptivity ($\epsilon = 26,900$) constants were used, given that the main pigment of the sample was not known.

Determination of kinetic parameters

The behaviour of anthocyanin degradation during ohmic and conventional processing was modelled using three different models: the zero and first order models and the Weibull model. After choosing the best model, three equations were used to describe the variance of the rate of a chemical reaction with temperature: Arrhenius equation, Ball model and Eyring model.

Zero order and first order model

As the degradation kinetics of most biological materials and colour of food systems follow the zero-order equation (Equation 3) or first-order equation (Equation 4) reactions (Maskan, 2006), both of these models were chosen to evaluate anthocyanin degradation during ohmic and conventional heating.

$$C_t = C_o - kt \quad \text{Equation 3}$$

$$C_t = C_o \exp(-kt) \quad \text{Equation 4}$$

In these equations, C_t was the anthocyanin concentration at a time t , C_o was the initial anthocyanin concentration (mg L^{-1}) and k was the degradation rate constant (min^{-1}). The parameter k gave the rate at which the degradation of the compound took place, the higher the k value the lower the stability of compound (Rawson *et al.*, 2012).

Weibull model

The Weibull model can be employed to describe chemical degradation kinetics using Equation 5 as follows:

$$C = C_o \cdot \exp[-b \cdot t^n] \quad \text{Equation 5}$$

In Equation 5, b (minutes^{-n}) and n were the shape and scale factors of the distribution curve, respectively. The n parameter indicated concavity (tail-forming) or convexity (shoulder-forming) of the curve when it took values below and above 1, respectively. As reported by Corradini and Peleg (2004) because the slope of this curve has rate units, Equation 5 might be considered a kinetic model. In this case, the familiar first-order kinetics was a particular case in which $n = 1$.

Arrhenius and Ball models

Using degradation rate constant (k) obtained for the first order model, the decimal reduction time (D -value) and the half-life value of degradation ($t_{1/2}$) were determined using Equations (6) and (7), in that order, for each treatment and temperature analysed:

$$D = \frac{\ln(10)}{k} \quad \text{Equation 6}$$

$$t_{1/2} = \frac{\ln(2)}{k} \quad \text{Equation 7}$$

The thermal resistance coefficient (z -value) was determined to compare ohmic and conventional heating. This coefficient is the temperature needed to vary D -value one log unit, and it was obtained using the Ball model (Equation 8):

$$z = -(T - T_{ref}) / (\log D - \log D_{ref}) \quad \text{Equation 8}$$

The effect of the temperature on k could be also evaluated by applying the Arrhenius equation as follows:

$$k = k_{Ref} \exp \left[\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{Ref}} \right) \right] \quad \text{Equation 9}$$

In Equation 9, T_{Ref} was the reference temperature (78.8°C), k_{Ref} was the reaction rate constant at T_{Ref} (min^{-1}), E_a was the activation energy (J mol^{-1}), R was the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T was the temperature (K). Equation 9 was used to calculate the activation energy (E_a) for ohmic and conventional process.

Eyring model

The Eyring equation (Equation 10) also describes the temperature dependence of reaction rate:

$$k = \frac{k_B}{h} e^{-\frac{\Delta H^\ddagger}{RT}} \cdot e^{\frac{\Delta S^\ddagger}{R}} \quad \text{Equation 10}$$

or

$$\ln \frac{k}{T} = \frac{\Delta H^\ddagger}{R} \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R} \quad \text{Equation 11}$$

In Equation 10, k was the rate constant of the overall reaction, h was the Planck's constant ($6.6262 \times 10^{-34} \text{ J s}$), k_B was the Boltzmann's constant ($1.3806 \times 10^{-23} \text{ J K}^{-1}$), ΔH^\ddagger was the activation enthalpy (kJ mol^{-1}) and ΔS^\ddagger was the activation entropy ($\text{J mol}^{-1} \text{ K}^{-1}$).

A plot of $\ln(k/T)$ versus $1/T$ (Eq. 11) produced a straight line in which the slope was $\Delta H^\ddagger/R$. Additionally, from the thermodynamics, the free activation enthalpy (ΔG^\ddagger , kJ mol^{-1}) and activation entropy (ΔS^\ddagger) could be calculated using Equations 12 and 13, respectively:

$$\Delta G^\ddagger = -R \cdot T \cdot \ln \left(\frac{k \cdot h}{k_B \cdot T} \right) \quad \text{Equation 12}$$

$$\Delta S^\ddagger = \frac{\Delta H^\ddagger - \Delta S^\ddagger T}{T} \quad \text{Equation 13}$$

A comparison with Equations 9 and 11 showed that E_a and ΔH^\ddagger or $\ln k_{ref}$ and ΔS^\ddagger were analogous quantities. These two energies are frequently used interchangeably in the literature to define the activation barrier of a reaction.

Statistical analysis and experimental design

The effect of temperatures and processes used on anthocyanin content in blackberry pulp was studied. Experiments were performed in duplicate, and analyses in triplicate. Also, fresh pulps were analysed before every batch. The estimation of kinetic parameters was carried out using nonlinear estimation and Statistica 13.0 (Statsoft Inc., Tulsa, USA). The average errors between the experimental values and those predicted by the models were calculated by Equation 14 as follows:

$$E(\%) = \frac{100}{m} \sum_{i=1}^n \left| \frac{y_{exp} - y_{pred}}{y_{exp}} \right| \quad \text{Equation 14}$$

In Equation 14, E was the average error, m was the number of experimental data points, y_{exp} were the experimental values and y_{pred} were the values predicted by the model.

The goodness of fit of the proposed models for degradation kinetics data was estimated by means of the determination coefficient (R^2), the Chi-squared parameter (χ^2) and the standard error of means ($S.E.M.$). Chi-squared and $S.E.M.$ parameters were calculated by Equations 15 and 16, respectively. p was the number of parameters.

$$\chi^2 = \frac{\sum (y_{exp} - y_{pred})^2}{(m - p)} \quad \text{Equation 15}$$

$$S.E.M. = \frac{\sum (y_{exp} - y_{pred})^2}{\sqrt{m}} \quad \text{Equation 16}$$

Results and discussion

Monomeric anthocyanin degradation

The average total monomeric anthocyanin content of the fresh blackberry pulps was of $281 \pm 12 \text{ mg L}^{-1}$. Overall, literature shows higher values for total anthocyanins in blackberry fruits (Kaume *et al.*, 2012) and juices (Wang and Xu, 2007). However, a similar anthocyanin content was found by Acosta-Montoya *et al.* (2010) for blackberries in the second stage of ripeness. Higher values obtained by other authors could be related to the anthocyanin extraction procedure. The fresh sample was assayed to verify the effect of the heating phase on anthocyanins degradation. In all cases, the concentration after the heating phase (time zero of the kinetic curve) was slightly higher than the concentration in the fresh sample ($459 \pm 35 \text{ mg L}^{-1}$). This result shows that the heating time (time needed to reach the holding temperature) was not long enough to promote degradation of the compound. Moreover, heating probably contributed to a better extraction of the compound from the cellular tissue.

Anthocyanin degradation tendency with time for different temperatures during treatment by ohmic and conventional heating is shown in Fig. 1. It is possible to observe that high temperatures and long heating times can negatively affect the nutritional quality of the final product. It is important to point out that, in the present work, the heating curve was equal in both experiments, which allowed the analysis of the non-thermal effects of the electric field applied. Fig. 1(a) shows the anthocyanin degradation at 70 and 75°C, while Fig. 1(b) at 80 and 90°C. Anthocyanin degradation was observed in all experiments. However, as expected, degradation was less pronounced when lower temperatures were used (70 and 75°C). At these temperatures, the maximum observed degradation, after 90 min, was lower than 20%. When higher temperatures were applied (80 and 90°C), the degradation, following processing for 90 min, was up to 40%. Moreover, Fig. 1(b) also shows that when higher voltages were applied (125 V), degradation was greater. This suggests that not only temperature, but also the electric field had an influence on the anthocyanins present in the blackberry pulp. These results show that the choice of the method of heating was a relevant variable in the present work of thermal degradation of anthocyanins.

Kinetic modelling

Three different models were fitted to the experimental data: zero order, first order and Weibull distribution. Zero order reaction was used

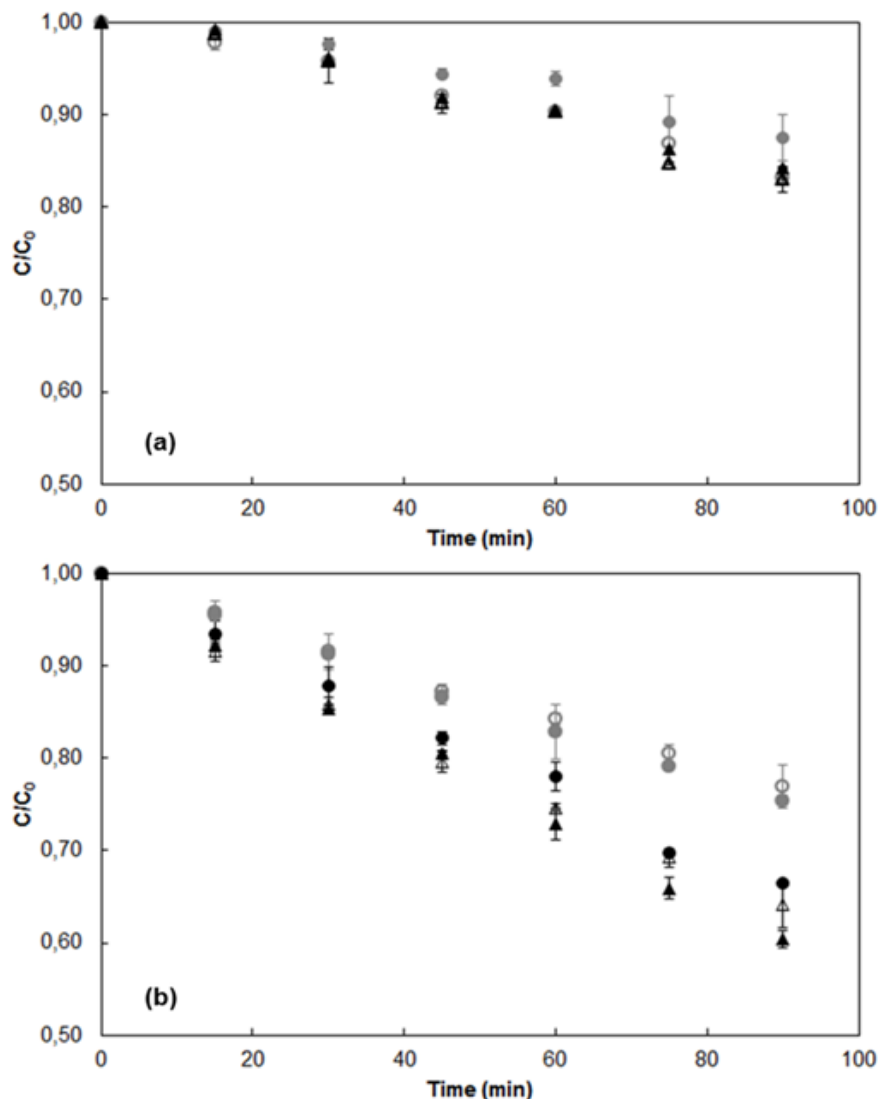


Fig 1. Anthocyanin degradation as function of time treated at different temperatures, using ohmic heating (full symbols) and conventional heating (empty symbols); (a) 70°C (circles) and 75°C (triangles), (b) 80°C (grey circles), 80°C and 125V (black circles) and 90°C (triangles).

by Alighourchi and Barzegar (2009) to evaluate the anthocyanin degradation during the storage of pomegranate juice, and by Zheng and Lu (2009) to predict the retention of ascorbic acid, total phenols and antioxidant activity during the storage of pasteurised pineapple juice. First order models have been widely used to describe nutrient degradation; for anthocyanins this model has been applied for different products, such as a model juice (Harbourne *et al.*, 2008), blackberry juice and concentrate (Wang and Xu, 2007) and strawberry juice (Garzon and Wrolstad, 2002). The Weibull model has also been employed to describe chemical degradation kinetics in fruit products (Manso *et al.*, 2001; Odriozola-Serrano *et al.*, 2009; Oms-Oliu *et al.*, 2009). The adequacy of each model was evaluated using the parameters presented in Table 1.

Table 1. Parameters used to evaluate the performance of selected models to describe anthocyanin degradation.

Model (equation)	R^2	χ^2	$S.E.M.$	E (%)
Zero order (3)	[0.941; 0.99]	[4.8460; 39.2677]	[3.0649; 60.0266]	[2.32; 40.22]
First order (4)	[0.933; 0.999]	[0.0001; 0.0001]	[0.000004; 0.00018]	[0.18; 1.14]
Weibull distribution (5)	[0.980; 0.999]	[0.0001; 0.0001]	[0.000003; 0.0001]	[0.19; 0.81]

All models presented values close to 1 for the determination coefficient (R^2). However, the zero order model presented higher values for the Chi-squared parameter (χ^2), the standard error of means ($S.E.M.$) and the average error (E), and, for this reason, this model was rejected. Both first order and Weibull models had a good fit, presenting reasonably low values for χ^2 , $S.E.M$ and E . In the present work,

the first order was used to model the results and obtain the degradation rate constant (k) due to its mathematical simplicity: it had one less parameter than the Weibull distribution, and offered similar goodness of fit. Table 2 shows the parameters of the first-order model obtained for each temperature and technology evaluated; the k values ranged from 0.0155 to 0.0051 min^{-1} . Similar k values were found by Cisse *et al.* (2009) using a first order model to fit the data for anthocyanin degradation in blackberry juice.

The rate of degradation, k , was significantly lower for the experiments performed at 70 and 75°C. When 80°C was employed, there was a significant increase in the k value. Moreover, at the highest temperature studied, 90°C, the rate of degradation observed was the highest. For the same temperature, there was no difference between samples processed using conventional or ohmic heating at 23 V ($p > 0.05$). Conversely, when ohmic heating using 125 V was applied, the rate of degradation at 80°C was higher than the observed for both the conventional and the ohmic heating using lower voltages (23 V) at the same temperature.

Arrhenius and Ball models

The decimal reduction (D -values) and the half-time ($t_{1/2}$) values for each experiment are shown in Table 2; these values represent the time necessary to degrade 90 and 50% of the pulp anthocyanins, respectively. The D -values varied from 466 to 1,228 min for conventional heating, and from 427 to 1,484 min for ohmic heating. The half-time was between 140 and 370 min for the conventional treatment, and 129 and 447 min for the ohmic treatment. Overall, both parameters decreased as the temperature of the process increased, which was in agreement to the greater k values of these processes. The z value, shown in Table 2, indicates the number of degrees the temperature has to be increased to achieve a ten-

fold reduction of the D value. For the ohmic heating process, z was 35.3°C, while for conventional processing it was 44.7°C. The values were close, meaning that the reaction showed similar temperature dependence for both heating technologies.

For the treatment at 80°C using 125 V, both D and $t_{1/2}$ parameters were smaller than the other treatments at the same temperature. These results corroborate the findings that the presence of a low voltage electric field (23 V) did not affect the anthocyanin degradation (Mercali *et al.*, 2013; 2015). However higher voltage electric field interfered in the manner anthocyanins reacted in the medium.

Studies suggest that higher phenolic compound and vitamin degradation rates during ohmic heating could be related to distinct non-thermal effects. Assiry *et al.* (2003) found that, during ohmic heating of a buffer solution of pH 3.5, power, temperature and NaCl content affected the degradation rate of ascorbic acid. According to the authors, the kinetics of degradation was described adequately by a first-order model, but electrode reactions and electrolysis products might influence on the kinetics parameters. Sarkis *et al.* (2013b) evaluated anthocyanin degradation in blueberry pulp during thermal treatment by ohmic and conventional heating and the results showed that high voltage gradients during ohmic heating induced greater degradation rates due to electrochemical reactions. The researchers also found that anthocyanin degradation was significantly influenced by the heating voltage (120 – 200 V). Electrode reactions, electrolysis of the solution, as well as reactions between electrode materials and the electrolysis products, might influence the reaction mechanism and the kinetic parameters. According to Cisse *et al.* (2009), anthocyanins are very reactive toward metals. They might form complexes with iron, which can be released in the medium due to electrode corrosion.

Table 2. Parameters of the first-order model for anthocyanin degradation in blackberry pulp during ohmic and conventional heating.

Process	T (°C)	k (min^{-1})*	D (min)*	$t_{1/2}$ (min)*	z (°C)
Conventional heating	70	0.0019 ± 0.0001 ^a	1228 ± 56 ^b	370 ± 17 ^b	35.33 ± 1.46
	75	0.00197 ± 0.00001 ^a	1168 ± 4 ^b	351 ± 1 ^b	
	80	0.0029 ± 0.0003 ^b	794 ± 72 ^c	239 ± 22 ^c	
	90	0.0049 ± 0.0002 ^{cd}	466 ± 17 ^d	140 ± 5 ^d	
Ohmic heating	70	0.00155 ± 0.0003 ^a	1484 ± 31 ^a	447 ± 9 ^a	44.70 ± 0.47
	75	0.00183 ± 0.00002 ^a	1259 ± 15 ^b	376 ± 15 ^b	
	80	0.0031 ± 0.0001 ^b	737 ± 29 ^c	222 ± 9 ^c	
	90	0.0051 ± 0.0002 ^c	427 ± 15 ^d	129 ± 5 ^d	
Ohmic heating (125 V)	80	0.0043 ± 0.0004 ^d	540 ± 55 ^d	162 ± 17 ^d	

* Results are the average values of two independent experiments (mean ± standard error); means in the same column with different lowercase letters are significantly different ($p < 0.05$).

In the present work, despite using platinum electrodes, electrolysis reactions were observed at low intensity. Gas production appeared to occur above 40°C. The presence of stainless steel temperature sensors, the use of a high voltage (125 V) and the use of a low-frequency electric current (60 Hz) might have contributed to the occurrence of these reactions. Qihua *et al.* (1993) also observed bubble formation during the heating process, probably because of electrochemical reactions, especially when the orange juice temperature reached 50°C. This might explain the results obtained in the present work: products from electrochemical reactions released in the medium might catalyse the degradation pathways involving oxygen. The occurrence of electrolysis could be minimised using more adequate materials for the temperature sensors and applying ohmic heating at higher frequencies. Mercali *et al.* (2014) reported that above 100 Hz these reactions were reduced, and as a result, ohmic and conventional heating processes showed similar degradation rates of ascorbic acid and similar colour changes.

The activation energy (E_a), shown in Table 3 was obtained by fitting the data into the Arrhenius equation. This parameter is an excellent indicator of the sensitivity of the reaction to temperature. For the ohmic heating process, E_a was 67 kJ mol⁻¹, while for conventional processing; it was 56 kJ mol⁻¹. The aforementioned values were close, which indicates that the temperature dependence of the reaction was similar for both heating technologies. It is noteworthy that the experiment conducted with 125 V (OH, 80°C) was not taken into account to estimate the activation energy. Cisse *et al.* (2009) reported values of activation energies of 66 and 37 kJ mol⁻¹ for blood orange and blackberry juices, respectively. They also evaluated four different roselle extracts, and found values between 47-61 kJ mol⁻¹. Mercali *et al.* (2015) found E_a values of 75.5 and 73.9 kJ mol⁻¹ for anthocyanin degradation of jaboticaba juice during ohmic and conventional heating (temperature range 70-90°C), respectively.

Table 3. Parameters of the Arrhenius equation obtained for anthocyanin degradation during ohmic and conventional heating.

Process	E_a (kJ mol ⁻¹)	k_{Ref} (min ⁻¹)*	R^2
Conventional heating	56 ± 1	0.0027 ± 0.0001	0.983
Ohmic heating	67 ± 2	0.0027 ± 0.0001	0.986

* T_{Ref} = 78.8°C

Eyring model

The Eyring equation is an alternative model to describe the temperature dependence of reaction rate. It considers that there is an energy barrier on the pathway between the reactants and the product. The barrier determines a 'threshold energy' or minimum energy necessary to permit the reaction to occur. This equation follows the transition state theory, which states that there is a thermodynamic equilibrium between the transition state and the state of reactants at the top of the energy barrier. Moreover, it assumes that the rate of chemical reaction is proportional to the concentration of the particles in the high-energy transition state. Table 4 shows the parameters obtained from the Eyring model for all temperatures and type of heating technology evaluated (except for OH with 125 V).

ΔH^\ddagger is the difference between the enthalpy of the transition state and the sum of the enthalpies of the reactants in the ground state. As shown in Table 4, ΔH^\ddagger values were similar for all conditions, varying between 53.6 and 64 kJ mol⁻¹. This parameter is equivalent to the energy of activation (E_a) of the Arrhenius equation. Both parameters are frequently used in the literature to define the activation barrier of a reaction. Cisse *et al.* (2009) reported lower values of ΔH^\ddagger (34.48 kJ mol⁻¹) for anthocyanin degradation of blackberry pulp under heating using temperatures ranging from 30 – 100°C.

ΔG^\ddagger might be considered to be the driving force of a chemical reaction, and determines the extent and spontaneity of the reaction; it showed very close values for all conditions evaluated in the present work (102.3 - 105.5 kJ mol⁻¹). The positive sign means that anthocyanin degradation is a non-spontaneous reaction. ΔS^\ddagger showed negative values, ranging from -114 to -144 J mol⁻¹ K⁻¹. Cisse and others (2009) found values of -233 J mol⁻¹ K⁻¹ for blackberry pulp heated in temperatures between 30 and 100°C. This parameter gives the difference between the entropy of the transition state, and the sum of the entropies of the reactants. The negative values indicate that the activated complex in the transition state has a more ordered or more rigid structure than the reactants in the ground state. Table 4 shows that ΔS^\ddagger was similar for both heating technologies in all temperatures, which indicates similar reactivity and reaction times.

The results of the present work are in agreement with those found on anthocyanin degradation in acerola and jaboticaba pulp (Mercali *et al.*, 2014; Mercali *et al.*, 2015). In those studies, ohmic heating was applied using low voltages (25 V), and all kinetic and thermodynamic parameters evaluated showed similar values for both technologies.

Table 4. Parameters of the Eyring equation obtained for anthocyanin degradation during ohmic and conventional heating.

Process	T (°C)	ΔH^\ddagger (kJ mol ⁻¹)	ΔG^\ddagger (kJ mol ⁻¹)	ΔS^\ddagger (J mol ⁻¹ K ⁻¹)
Conventional heating	70	53.5 ± 0.6	102.3 ± 0.1	-142 ± 1
	75	53.5 ± 0.6	103.7 ± 0.01	-144 ± 2
	80	53.4 ± 0.6	104.1 ± 0.3	-144 ± 1
	90	53.3 ± 0.6	105.5 ± 0.1	-144 ± 1
Ohmic heating	70	64.0 ± 2.0	102.9 ± 0.1	-114 ± 4
	75	64.0 ± 2.0	103.9 ± 0.04	-115 ± 5
	80	64.0 ± 2.0	103.9 ± 0.1	-114 ± 5
	90	64.0 ± 2.0	105.3 ± 0.1	-114 ± 5

Conclusions

The present work evaluated the effect of ohmic heating on anthocyanin stability of blackberry pulp. The first order model, the Arrhenius equation, the Ball and Eyring models were suitable for predicting anthocyanin degradation during heating. For low voltage (23 V), ohmic and conventional heating technologies showed similar values for all kinetic parameters (when compared to similar temperature), indicating that anthocyanins had similar degradation pathways during ohmic and conventional heating. These results reinforce the finding that the presence of the low voltage electric field does not affect the mechanism and the rate of anthocyanin degradation. However, high voltages associated with low frequency increased anthocyanin degradation which might probably be due to the occurrence of electrochemical reactions. This outcome underlines that special care must be taken when choosing process variables. When using high voltages, it is important to choose high electrical field frequencies and suitable materials for electrodes and sensors. Ohmic heating is an alternative thermal technology appropriate for thermal processing of fruit pulps and products if the variables of the process are properly chosen.

Acknowledgement

The authors acknowledge the financial support received from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasil), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil), and Mais Fruta Company for supplying the blackberry pulp.

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