

# EFFECTS OF TEMPERATURE IN DEGRADATION KINETICS OF ANTHOCYANIN FROM JAMUN FRUIT (EUGENIA JAMBOLANA) USING THE ARRHENIUS, EYRING AND BALL MODELS

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Anthocyanins are flavonoids found in fruits and vegetables, ranging from red to violet and blue, which are shown to be a potential substitute for artificial colors, especially in food. Due to its thermal instability, this work had as objective to establish the kinetic and thermodynamic parameters that describe the thermal degradation of the anthocyanins from jamun fruit between 50 and 80 ° C using the Arrhenius, Eyring and Ball models. The thermal degradation process of the anthocyanins followed a model of first-order kinetics and the three models correlated the temperature to the degradative process coefficients of determination between 0.939 and 0.960. The activation energy was 93.383 kJ.mol<sup>-1</sup> and, thermodynamically, the values of  $\Delta H$ ,  $\Delta S$  and  $\Delta G$  did not differ when using the Arrhenius or Eyring approaches, allowing, according to both, to characterize the degradation reaction as endothermic, non-spontaneous and with the transition state with less structural freedom than the reagents. According to the Ball model, the temperature range  $z$  was 27.03°C.

*KEY WORDS: THERMODYNAMIC, CYANIDIN, ENTHALPY, NATURAL PIGMENT.*

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## 1 INTRODUCTION

Artificial pigments have been favored for at least the last 100 years (PATIL et al., 2009). However, recognition of how harmful they may be to human health has made researchers make natural pigments an alternative to consumers whose desire for a healthy diet is growing (WU et al., 2014). Thus, natural colorants with attractive colors and potential beneficial health action have aroused interest in obtaining and making them available (ROSSI, 2008).

Among these natural dyes, we highlight the anthocyanins, phenolic compounds that are widely distributed in fruits and vegetables, source of a range of colors in nature, such as red, blue, purple and orange (AHMADIANI et al., 2014). Due to its association with beneficial properties, such as antioxidant activity, cardioprotective capacity (HE, GIUSTI, 2010), anti-inflammatory effects (LEE, FRANCIS, 2011) and anticancer effects, mainly related to the colon (NORBERTO et al., 2013, SANTOS et al. al., 2013), there has been considerable interest in anthocyanins in recent years (OLIVEIRA et al., 2015).

Jamun (*Eugenia jambolana*) is a fruit from *Mirtáceaes* family, of purple color when ripe, astringent flavor and are an important source of phenolic compounds like anthocyanins (SHAHEER et al., 2014). Jambolão trees have a very high production of which a part is used *in natura* form by the population, with a short useful life, while a great part ends up being wasted mainly by the lack of use of the processed fruit (LAGO; GOMES; SILVA, 2006).

Due to the bright and attractive colors of pulp and bark (characteristics of anthocyanins) and because they are non-toxic and soluble in water, jamun fruits has aroused interest in incorporation into aqueous systems by making a natural food coloring dye. This way we can use the wasted fruits for an industrial use (PEREIRA, MUSSI, 2015). However, for the food industry, the thermal instability of anthocyanins is a problem and the kinetics of degradation depends mainly on the specific composition of the anthocyanins and the characteristics of the food matrix (CISSE et al., 2009).

Cisse et al. (2012) and Harbourne et al. (2008), among other authors, reported that the first-order model best describes the thermal degradation kinetics of fruit extracts, especially rich in anthocyanin. To describe the dependence between the kinetic degradation constant and the temperature, the Arrhenius model has been a reference because it presents results consistent with the experimental measurements in the various areas of knowledge where it is used (SILVA, 2013). However, the Eyring (or Eyring-Polanyi) and Ball models are alternatives that also make it possible to describe this same relationship of dependence for anthocyanins. The first one is based on the theory of transition state, whose parameters are the entropy and entropy of activation, while the second follows the approach used in food processing to describe the destruction of microorganisms, using as parameters the time of reduction decimal and z-factor, temperature interval that causes a ten-fold variation in the rate of degradation (CISSE et al., 2009).

Thus, the objective of this work was to compare Arrhenius' classical approach with the Eyring and Ball approaches, in the description of the temperature dependence with the thermal degradation constant of anthocyanins from jamun fruit between 50 and 80°C.

## 2 MATERIALS AND METHODS

### 2.1 PIGMENT EXTRACTION

Jamun fruits were collected in Santo Antônio da Patrulha - RS, south of Brazil, were packed in plastic bowl and frozen at -11°C in the Laboratory of Kinetics and Thermodynamics of Degraded Processes (LCTPD - FURG). Anthocyanins from jamun fruit were extract as described by Drunkler (2003), using 70% ethanol as the solvent (ratio 1:2 w/v). After extraction, the extract was vacuum fi ltered and stored refrigerated ( $5.0 \pm 1.0^\circ\text{C}$ ) in amber bottles. The extraction was performed in a

single batch in order to ensure that the same extract was used in all assays of thermal degradation, ensuring the same initial characteristics.

## 2.2 THERMAL DEGRADATION

For processing between 50 and 80°C, 5 mL samples of the anthocyanin extract were put into screw capped test tubes and placed in thermostatic baths (Oxylab OXY 208 Batch), protected from light. The samples were collected periodically, in triplicate, as per Peron et al. (2017) until half of the initial anthocyanin concentration was reached for each temperature condition explored. The pH of the extract was adjusted to 3.0 by the addition of 1 M HCl (Kasvi pH meter K39-2014B) to ensure the stability of the anthocyanins (Castellar et al., 2003). The initial anthocyanin concentration ( $C_{AO}$ ) and the concentration immediately after sample collection ( $C_A$ ) were determined for each temperature and the results were expressed as  $C_{AO}/C_A$ .

## 2.3 ANTHOCYANIN CONCENTRATION

The anthocyanin concentration ( $C_A$ ) was monitored spectrophotometrically (Bel Photonics Spectrophotometer SP 1105) using the absorbance at 535 nm and calculated according to Fuleki; Francis (1968), according to Equation (1):

$$C_A = \left( \frac{Abs_{535} \times M_{\text{cian-3-glicosideo}} \times D}{\epsilon} \right) * 100 \quad (1)$$

where  $C_A$  is the concentration of total anthocyanins expressed in mg of cyanidin-3-glycoside.100g de amostra<sup>-1</sup>,  $Abs_{535}$  is the absorbance of the extract read at 535 nm,  $PM_{\text{cian-3,5-glicosideo}}$  is the molar mass of cyanidin-3 -glucoside equal to 449.2 g mol<sup>-1</sup>,  $FD$  is the dilution factor and  $\epsilon$  is the molar extinction coefficient of cyanidin-3-glycoside in ethanol solution acidified at 535 nm, whose value is 26,900 L mol<sup>-1</sup>cm<sup>-1</sup>. All analyzes were performed in triplicate. The extract had its pH adjusted to 3 with 0.1 M HCl, measured in bench pHmeter (Model K381014B, Kasvi), which guarantees greater stability to anthocyanins.

## 2.4 DEGRADATION RATE CONSTANT

The degradation rate constant of the anthocyanin extract and  $k_d$  (h<sup>-1</sup>) were estimated according to Equation 2 (Fogler, 2012), assuming first order reaction kinetics:

$$\ln \frac{C_{AO}}{C_A} = k_d \times t \quad (2)$$

where  $C_{AO}$  e  $C_A$  are the initial anthocyanin concentrations and at a given time in mg of cyanidin-3,5-glycoside.100g of sample<sup>-1</sup> and  $t$  is the time in h.

## 2.4 ARRHENIUS, EYRING AND BALL MODELS

The models of Arrhenius, Eyring and Ball, Equations (3), (4) and (5) were used to describe the behavior of the thermal degradation reaction of anthocyanins as a function of temperature (CISSE et al., 2009).

$$k_d = A \times e^{\frac{-E}{R}} \quad (3)$$

where  $k_d$  is the degradation rate constant in  $h^{-1}$ ,  $A$  is the frequency factor in  $h^{-1}$ ,  $E_a$  is the activation energy of the degradation reaction in  $kJ\ mol^{-1}$ ,  $T$  is the temperature in  $K$  and  $R$  is the gas constant in  $kJ\ mol^{-1}K^{-1}$ .

$$k_d = \frac{k_B}{h} T \times e^{-\frac{\Delta H - T\Delta S}{R}} \quad (4)$$

where  $k_B$  is the Boltzmann constant ( $1.381 \times 10^{-23}\ J\ K^{-1}$ ),  $h$  is the Planck constant ( $6.626 \times 10^{-34}\ J\ s$ ),  $\Delta H$  is the activation enthalpy ( $J\ mol^{-1}$ ) and  $\Delta S$  is the activation entropy in  $J\ mol^{-1}K^{-1}$

$$D = h \left( \frac{D_0}{k_d} \right) = D_0 \times 10^{-T/z} \quad (5)$$

where  $D$  is the decimal reduction time in  $h$ ,  $T$  is the temperature in  $^{\circ}C$  and  $z$  is the temperature range in  $^{\circ}C$ , which causes a 10-fold variation in speed of degradation.

The parameter  $z$  is determined by fitting the experimental data to the model provided by Equation (6) (FONTAN et al., 2012; MERCALI et al., 2015).

$$\log D = \frac{1}{z} \times T + B \quad \log D = \frac{1}{z} \cdot T + B \quad (6)$$

where  $T$  is the temperature in  $^{\circ}C$  and  $B$  is the linear coefficient, provided by the line equation.

## 2.5 THERMODYNAMIC PARAMETERS

The activation enthalpy  $\Delta H$  ( $J\ mol^{-1}$ ), for the Arrhenius approach, as well as the activation entropy  $\Delta S$  ( $J\ mol^{-1}K^{-1}$ ), and the free energy of inactivation  $\Delta G$  ( $kJ\ mol^{-1}$ ), Equations (6), (7) and (8) are determined according to Cisse et al. (2009) and Bastos; Rodrigues; Souza (2011).

$$\Delta H = E - R \times T \quad (6)$$

$$\Delta G = -R \times T \ln \left( \frac{k_d \times h}{k_B \times T} \right) \quad (7)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (8)$$

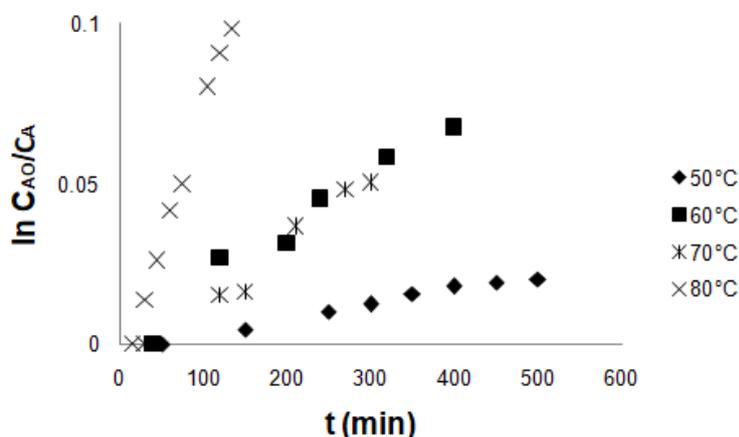
## 2.6 STATISTICAL ANALYSIS

Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test (5% confidence level) using *Statistica* (data analysis software system) version 7.0 for Windows.

## 3 RESULTS AND DISCUSSION

The regression of the data evidenced the adjustment of the first order kinetic model for the degradation of the anthocyanins from jamun fruit (Figure 1), with determination coefficients of 0.989 ( $50^{\circ}C$ ), 0.961 ( $60^{\circ}C$ ), 0.974 ( $70^{\circ}C$ ) and  $80^{\circ}C$  (0.995). According to Levenspiel (2000) the reaction is considered of first order when the speed is directly proportional to the concentration of the reagent. Similar results were found by Mercali et al. (2005), who also described the first order kinetic model in their study of degradation of anthocyanin from Jabuticaba between  $70$  and  $90^{\circ}C$ , as well as Hou et al. (2013), for the degradation of black rice isolated anthocyanins between  $80$  and  $100^{\circ}C$ .

**FIGURE 1. DEGRADATION OF ANTHOCYANIN FROM JAMUN FRUIT DURING HEATING BETWEEN 50 AND 80°C AT PH 3. EACH POINT REPRESENTS THE AVERAGE OF 3 REPLICATES AND THE STANDARD ERROR OF EACH POINT IS AROUND 5%.**

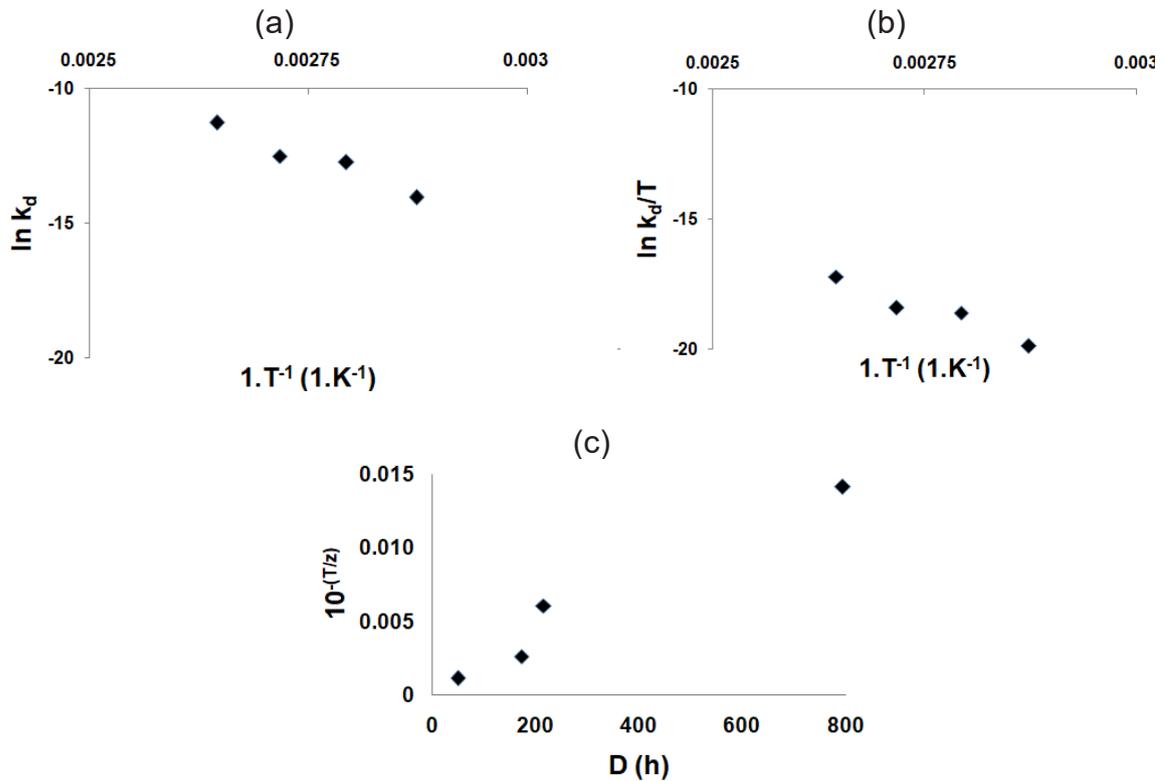


The increase in temperature caused an increase of the degradation process, evidenced by the values of  $k_d$  obtained, as expected. Comparing the two extremes of temperature, at 50°C the value of  $k_d$  was significantly lower than that obtained at 80°C, whereas for the half-life the behavior was the inverse (Table 1). Thus, the lower the temperature, the greater the stability of the anthocyanins and consequently the longer the time required for the 50% reduction of the initial concentration to occur. This behavior has already been observed by Kara; Erçelebi (2013) for the anthocyanins from *Urmu mulberry* (*Morus nigra* L.) between 60 and 80°C, when they found that there was a reduction in anthocyanin concentration and consequently the half-life, as the temperature increased. The values obtained were 8.3, 4.4 and 3.2 h for 60, 70 and 80°C, respectively.

The comparison between the values obtained for the half-life of the anthocyanins from jamun fruit and from *Urmu mulberry*, rich in cyanidin-3-glycoside, also suggests a higher thermostability of the first extract. Similarly, the anthocyanins from jamun fruit are more thermostable than those extracted from purple corn at 70 and 80°C, whose half-life reached were 11.6 and 9 h (YANG et al., 2008).

The temperature dependence with the thermal degradation of the anthocyanins from jamun fruit extract, through  $k_d$  and  $D$ , was verified through three models: Arrhenius, Eyring and Ball. According to the determination coefficients obtained, the three models studied allowed the correlation between the temperature and the degradation rate constant and the decimal reduction time (Figure 2). The Arrhenius model is based on the collision theory, the classical approach used for chemical reactions, which explains the increase in the speed of reactions with increasing temperature, considering that the molecules, in order to react, have to collide with each other (CISSE et al., 2009). The Eyring model is based on the theory of transition state, whose rate of formation of products depends only on the rate of formation of a critical intermediary state from the reagents (MAHAN, 1974; DE PAULA; KEELER, 2007).

**FIGURA 2. ANTHOCYANIN DEGRADATION ACCORDING TO (A) ARRHENIUS MODEL (R2 = 0.943), (B) EYRING MODEL (R2 = 0.939) AND (C) BALL MODEL (R2 = 0.960).**



Through the Arrhenius model, the activation energy of the degradative process of anthocyanins from jamun fruit was determined: 93.38 kJ mol<sup>-1</sup>. The value obtained was higher than that achieved by Kara; Erçelebi (2013), 46.32 kJ mol<sup>-1</sup>, in the degradation of the anthocyanins of *Morus nigra* L., between 60 and 80°C and by Kopjar et al. (2009), 33.08 kJ mol<sup>-1</sup>, in the degradation of anthocyanins (control sample) of gooseberry juice between 30 and 90°C. Thus, anthocyanins from jamun fruit are more strongly temperature dependent than these other extracts, and the degradation reaction occurs slowly at low temperatures and relatively fast at high temperatures.

As well the Arrhenius model, the Eyring model allows the thermodynamic characterization of the degradative process. It considers that there is an energetic barrier in the path between the reagents and the product, which determines the minimum energy required for the reaction to occur. This model is based on the theory of the transition state and its parameters are enthalpy and entropy of activation (CISSE et al., 2009).

**TABLE 1 - KINETIC AND THERMODYNAMIC PARAMETERS OF ANTHOCYANIN DEGRADATION BETWEEN 50 AND 80°C, ACCORDING TO ARRHENIUS, EYRING AND BALL MODELS.**

Arrhenius Model					
T (°C)	$k_d$ (1.h <sup>-1</sup> )*	$t_{1/2}$ (h)	$\Delta H$ (kJ.mol <sup>-1</sup> )	$\Delta G$ (kJ.mol <sup>-1</sup> )	$\Delta S$ (J.mol <sup>-1</sup> K <sup>-1</sup> )
50	0.003 (0.986) <sup>a</sup>	239.016	90.490	126.274	-0.103
60	0.011 (0.961) <sup>b</sup>	64.780	90.406	126.101	-0.100
70	0.013 (0.974) <sup>c</sup>	51.727	90.323	129.019	-0.115
80	0.046 (0.995) <sup>d</sup>	14.964	90.240	128.711	-0.102
Eyring Model					
50	0.003 (0.986) <sup>a</sup>		88.474	123.631	-0.101
60	0.011 (0.961) <sup>b</sup>	239.016	88.474	123.462	-0.098
70	0.013 (0.974) <sup>c</sup>	64.780	88.475	126.319	-0.103
80	0.046 (0.995) <sup>d</sup>	51.727	88.474	126.017	-0.099
		14.964			
Ball Model					
T (°C)	$k_d$ (1.h <sup>-1</sup> )	D (h)	z (°C)	Do (h)	
50	0.003 (0.986) <sup>a</sup>	793.995			
60	0.011 (0.961) <sup>b</sup>	215.195			
70	0.013 (0.974) <sup>c</sup>	171.835	27.030	0,000017	
80	0.046 (0.995) <sup>d</sup>	49.710			

Numbers in parentheses are the coefficients of determination  
Equal letters in columns do not differ statistically ( $p < 0,05$ )

The values of  $\Delta H$ ,  $\Delta S$  and  $\Delta G$  (Table 1) did not differ significantly when using the Arrhenius and Eyring models. The activation enthalpy was positive, representing the endothermic state between the activated complex and the reagent. This parameter is equivalent to the activation energy that can be determined through the classical Arrhenius model and is used to determine the reaction activation barrier.

The Gibbs free energy of inactivation, considered the driving force of the chemical reaction, was positive indicating that the degradation reaction is non-spontaneous. However, negative activation entropy denoted that the transition state had a lower structural freedom than the reagents and similar values obtained indicate similar reaction times and reactivity times.

From the Ball model, the decimal reduction time was determined for each temperature and, as expected, was higher for the lower temperatures. As the temperature increases, the degradative process of the extract intensifies and the time to the reduction of 90% of its initial concentration decreases. The temperature range (z) suggests that anthocyanins from jamun fruit are less sensitive to heat than pathogenic microorganisms occurring in food. Luu-Thi et al. (2014) determined a z factor of 10.6°C for *Bacillus cereus* spores in a temperature range of 50 to 100°C and thus if the

jatropha anthocyanin extract was applied as a natural dye in a food product, for For example, these microorganisms would be eliminated before anthocyanin degradation, and food security would be maintained.

#### 4 CONCLUSIONS

The first-order model, as well as the three approaches - Arrhenius collision theory, Eyring's transition state theory and Ball's model - adequately described the thermal degradation of anthocyanins from jamun fruit between 50 and 80°C. Thermodynamically, the degradation reaction was characterized as endothermic, non-spontaneous and with a transition state more organized structurally than the regents. All of them can be easily used as tools to predict the loss of anthocyanins during processing at different temperatures. The kinetic and thermodynamic characterization of the process is one of the steps to elucidate the mechanisms of reaction during the heat treatment and thus to promote a greater stability of the extract, seeking to enable the anthocyanins to be used in industrial processes, especially in the food industry .

#### RESUMO

#### EFEITO DA TEMPERATURA NA CINÉTICA DE DEGRAÇÃO DAS ANTOCIANINAS DE JAMBOLÃO (*EUGENIA JAMBOLANA*) USANDO OS MODELOS DE ARRHENIUS, EYRING E BALL

As antocianinas são flavonóides encontrados em frutas e vegetais, de tonalidade que vão desde o vermelho ao violeta e azul, que se mostram como um potencial substituinte aos corantes artificiais, sobretudo nos alimentos. Dada a sua instabilidade térmica, este trabalho teve como objetivo estabelecer os parâmetros cinéticos e termodinâmicos que descrevem a degradação térmica das antocianinas de jambolão entre 50 e 80°C utilizando os modelos de Arrhenius, Eyring e Ball. O processo termodegradativo das antocianinas seguiu um modelo de cinética de primeira ordem e os três modelos correlacionaram a temperatura ao processo degradativo coeficientes de determinação entre 0.939 e 0.960. A energia de ativação foi de 93.383 kJ.mol<sup>-1</sup> (R<sup>2</sup> = 0,943) e, termodinamicamente os valores de  $\Delta H$ ,  $\Delta S$  e  $\Delta G$  não diferiram quando utilizadas as abordagens de Arrhenius ou Eyring, permitindo segundo ambos, caracterizar a reação de degradação como endotérmica, não-espontânea e com o estado de transição com uma liberdade estrutural inferior a dos regentes. De acordo com o modelo de Ball, o intervalo de temperatura  $z$  foi de 27.03°C.

**Palavras Chave:** Termodinâmica; Cianidina; Entalpia; Corantes naturais.

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