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Thermal degradation of the anthocyanins extracts from jabuticaba peels and red cabbage leaves

Degradação térmica de extratos antociânicos das cascas de jabuticaba e das folhas de repolho roxo

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Anthocyanins are phenolic compounds with tinctorial capacity. Their successful extraction and purification depends on information associated with their thermal stability. This work presents a study on the degradation kinetics of the anthocyanins in the peels of the jabuticaba fruits and the leaves of red cabbage through assays performed at different temperatures (ranging from 323.15 K to 363.15 K) and degradation times (up to 10 hours for jabuticaba and 30 hours for red cabbage). Total monomeric anthocyanins were determined through the pH differential method. Regarding the extraction, it was observed a larger quantity of anthocyanins from red cabbage extracts, with an average value of 1.3774 $mg \cdot g_{db}^{-1}$, when compared to jabuticaba extracts, with an average value of 1.0479 $mg \cdot g_{db}^{-1}$. The results suggest that the anthocyanins in the red cabbage leaves are much more thermally stable than the anthocyanins in the jabuticaba peels. The experimental assays suggest that the thermal degradation reaction proceeds according to a first-order kinetics model for the anthocyanins in both sources under study. The experimental values were treated according to their thermodynamic relations, which indicated that the thermal degradation process of the evaluated anthocyanins is endothermic and not spontaneous, with similar values of 64.8 $kJ \cdot mol^{-1}$ for the red cabbage and 70.2 $kJ \cdot mol^{-1}$ for the jabuticaba.

Keywords: *Brassica oleraceae*, *Myrciaria cauliflora*, kinetics.

Antocianinas são compostos fenólicos que apresentam capacidade tintorial. Sua extração e purificação bem-sucedidas dependem de informações associadas à sua estabilidade térmica. Este trabalho apresenta um estudo da cinética de degradação das antocianinas presentes nas cascas da jabuticaba e nas folhas do repolho roxo com ensaios realizados a diferentes temperaturas (entre 323.15 K e 363.15 K) e tempos de degradação (até 10 horas para a jabuticaba e 30 horas para o repolho roxo). As antocianinas monoméricas totais foram determinadas pelo método de pH diferencial. Em relação a extração, foi observada uma maior quantidade de antocianinas no extrato de repolho roxo, com um valor médio de 1.3774 $mg \cdot g_{db}^{-1}$, quando comparado ao extrato de jabuticaba, com um valor médio de 1.0479 $mg \cdot g_{db}^{-1}$. Os resultados indicaram que as antocianinas presentes nas folhas do repolho roxo são muito mais estáveis termicamente do que as antocianinas presentes nas cascas da jabuticaba. Os ensaios experimentais indicaram que a reação de degradação térmica segue uma cinética de primeira ordem para as antocianinas presentes em ambas as fontes estudadas. Os valores experimentais foram tratados por relações termodinâmicas que indicaram que o processo de degradação térmica das antocianinas avaliadas é endotérmico e não espontâneo, com valores similares de 64.8 $kJ \cdot mol^{-1}$ para o repolho roxo e 70.2 $kJ \cdot mol^{-1}$ para a jabuticaba.

Palavras chave: *Brassica oleraceae*, *Myrciaria cauliflora*, cinética.

1. INTRODUCTION

The quality of food products and their visual appeal are directly related to color [1]. According to literature [2], color stands out as one of the most important sensory attributes of a food item. It is therefore assumed that the quality and quantity of pigments in a food product are essential for its commercial acceptance [3].

Although synthetic colorants have a lower cost of production, greater stability and greater tinctorial capacity, one aspect that should be strongly considered is the fact that every passing year the number of permitted synthetic additives for use in the food industry decreases [4]. The use of artificial colorants has been questioned by different segments of society, and together with the continuous negative publicity, this trend has increased the interest in colorants of natural origin.

The replacement of synthetic colorants by natural colorants has therefore been strongly advocated by consumers who seek products with positive effects on human health.

Anthocyanins stand out among the natural colorants. They make up the largest group of water-soluble pigments of the vegetable kingdom [5] and are part of the flavonoid class. These bio-compounds with antioxidant properties are attracting great interest in the food, pharmaceutical and cosmetic industry mainly because of their presence in many fruits and vegetables [6] and their antioxidant [7], anticarcinogenic [8, 9] and antiviral capacity [9].

Unfortunately, the extraction/purification of the pigment and its successful incorporation into food items is still in need of further studies, especially concerning the degradation kinetics of these pigments when they are subjected to light, temperature fluctuations and pH, among other factors [10].

The red cabbage (*Brassica oleracea* L.), a rich source of anthocyanins [11], has its consumption associated with the prevention of diseases [12]. The vegetable is characterized by its long shelf life and therefore, can be easily stored [13]. The use of organic solvents associated with the extraction capability and the stability of the extracts is a subject of importance [14].

The jabuticaba (*Myrciaria cauliflora* Mart.) is known as one of the richest sources of anthocyanins in Brazil [15]. Due to the content of sugar, organic acid and terpene, the fruit has a sweet and sub-acidic flavor [16]. Balisteiro et al. (2017) [17] and Hsu et al. (2016) [18] suggest that its extract may be used as an alternative for the treatment of hyperglycemia.

The application of mathematical models capable of interpreting the kinetic parameters of the thermal degradation of anthocyanins, such as the reaction order, reaction rate, activation energy, enthalpy, entropy and Gibbs energy, has become essential to predict the quality losses that can occur during the thermal processing of products containing anthocyanins [19, 20].

In line with the importance of developing studies related to the degradation of anthocyanins, this work seeks to extract anthocyanins from jabuticaba peels (*M. cauliflora*) and red cabbage leaves (*B. oleraceae*) using water as a solvent and subjecting the anthocyanin extracts to different temperatures as a function of time to evaluate their thermal degradation. The experimental results were submitted to models to determine the kinetic and thermodynamic degradation parameters.

2. METHODOLOGY

The jabuticabas and red cabbages were carefully cleaned with tap water and the parts in the fruits and vegetables rich in anthocyanin (jabuticaba peels and red cabbage leaves) compounds were separated. Subsequently, the materials were dried in an oven with air circulation for a period of 48 hours at a temperature of 313.15 K. After drying, the materials were ground in a food processor and separated into particles smaller than 0.833 mm using a 20-mesh sieve. The samples were then placed in portions of 5 grams in a sealed package and kept in an ULT freezer at 188 K until the performance of the extraction.

The extraction was carried out with a jacketed glass cell connected to a thermostatic bath. A ratio of 60 ml of solvent (distilled water) per gram of dry material was used. The extraction was performed for a period of 3 h at a temperature of 313.15 K. The mixture was submitted to stirring at a controlled temperature to complete the mass transfer. After filtering the solution, the anthocyanin extract was put in 4.5 ml amber polypropylene tubes and then submitted to thermal degradation.

The red cabbage extracts were subjected to degradation for 30 h, with an evaluation in duplicate each 10 h (including $t = 0$) at four temperatures distributed in the interval between 323.15 and 353.15 K. The jabuticaba peel extracts were subjected to degradation for 10 h, with an evaluation in duplicate every 2 h (including $t = 0$) at five temperatures distributed in the interval between 323.15 and 363.15 K. Total monomeric anthocyanins (TMA) were determined using the pH differential method [21].

The pH differential method is based on the structural transformation of the anthocyanin as a function of pH in two buffer solutions: potassium chloride with pH 1.0 (0.025 M) and sodium acetate with pH 4.5 (0.4 M). According to this method, the difference in absorbance of the solutions of pH 1.0 and 4.5 is directly proportional to the concentration of TMA. After dilution of the extract, aliquots containing anthocyanins are buffered and the absorbance of the samples is determined at

the wavelengths of 510 nm and 700 nm. Total monomeric anthocyanins are calculated on a dry base ($mg \cdot g_{db}^{-1}$) with reference to cyanidin-3-glucoside, as represented by Equation (1).

$$\mathrm{TMA} = \frac{\left(\left[(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}\right] \cdot MW \cdot V \cdot \mathrm{DF} \cdot 1000\right)}{(\varepsilon \cdot m \cdot 1)} \quad (1)$$

In equation (1), MW represents the molar mass of cyanidin-3-glucoside (449.2 $g.mol^{-1}$), DF is the dilution factor of the sample, ε is the molar extinction coefficient of cyanidin-3-glucoside (26.9 $L.mol^{-1}.cm^{-1}$), 1000 represents the conversion of g to mg, 1 is the path of the wave in the cuvette in cm, V is the volume of the extracted solution and m is the dry solid mass used in the extraction. The determinations were performed using a digital scanning UV/VIS spectrophotometer of the brand Fento, model Cirrus 80SA.

The experimental uncertainty of total monomeric anthocyanins (δTMA) was estimated by a propagation method [22]. It was assumed that the final uncertainty depends on each uncertainty of absorbance (δA) for both pHs and wavelengths, mass (δm), volume (δV) and partial derivatives of Equation 1 as a function of all dependent variables. In accordance with the method the calculation takes the form presented by Equation (2).

$$\delta\mathrm{TMA} = \left(\left(\frac{\partial \mathrm{TMA}}{\partial A_{pH1.0}^{510}}\right)^2 (\delta A_{pH1.0}^{510})^2 + \left(\frac{\partial \mathrm{TMA}}{\partial A_{pH1.0}^{700}}\right)^2 (\delta A_{pH1.0}^{700})^2 + \left(\frac{\partial \mathrm{TMA}}{\partial A_{pH4.5}^{510}}\right)^2 (\delta A_{pH4.5}^{510})^2 + \left(\frac{\partial \mathrm{TMA}}{\partial A_{pH4.5}^{700}}\right)^2 (\delta A_{pH4.5}^{700})^2 + \left(\frac{\partial \mathrm{TMA}}{\partial m}\right)^2 (\delta m)^2 + \left(\frac{\partial \mathrm{TMA}}{\partial V}\right)^2 (\delta V)^2\right)^{\frac{1}{2}} \quad (2)$$

Previous studies conducted in different food matrices [23–26] suggest that the thermal degradation kinetics of anthocyanins proceed as a first-order reaction, as shown by Equation (3). The concentration of anthocyanins C_t determined at a constant temperature after a degradation time t from a known initial concentration C_0 enables the determination of the reaction kinetics constant k. Knowing the kinetics constant of the reaction determines the half-life time according to Equation (4).

$$C_t = C_0 \cdot \exp(-k \cdot t) \quad (3)$$

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

The dependence of the thermal degradation of the anthocyanins with temperature can be represented by the Arrhenius equation, Equation (5), where k_0 is defined as a frequency factor, E_a is the activation energy, R is the universal gas constant and T is the assay temperature. To determine the effect of temperature on the desired kinetic parameters, the constants obtained in Equation (3) are linearized by plotting k as a function of the inverse of the temperature, making it possible to relate the frequency factor with the linear coefficient of the curve and the activation energy (E_a) with the angular coefficient of the curve.

$$k = k_0 \cdot \mathrm{e}^{-\frac{E_a}{RT}} \quad (5)$$

In addition to the activation energy, such quantities as the Gibbs energy of activation $\Delta G^{\ddagger}$, the activation enthalpy $\Delta H^{\ddagger}$ and the activation entropy $\Delta S^{\ddagger}$ provide important information about the molecular interactions during the thermal degradation. These quantities can be determined with the Eyring-Polanyi model [27–31] based on the transition-state theory and expressed by Equation (6).

$$k = \frac{k_B}{h} \cdot T \cdot \mathrm{e}^{-\frac{\Delta G^{\ddagger}}{RT}} \quad (6)$$

In Equation (6), k_B is the Boltzmann constant (1.38064852×10^{-23} J·K^{-1}) and h represents the Planck's constant ($6.626070040\times10^{-34}$ J·s). The Gibbs energy of activation is defined by the thermodynamic relationship presented by Equation (7), where, for a first-order reaction, the activation enthalpy can be determined as suggested in Equation (8).

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T \cdot \Delta S^{\ddagger} \quad (7)$$

$$\Delta H^{\ddagger} = E_a - R \cdot T \quad (8)$$

All statistical analyses were conducted with Microsoft Excel 2013. Results were expressed as means ± experimental uncertainty. The effects of time and temperature on concentration of TMA were determined by one-way ANOVA. The significant difference between means was evaluated by Tukey's post hoc test. The level of significance of all testes was 0.05.

3. RESULTS AND DISCUSSION

Tables 1 and 2 show the experimental concentrations of total monomeric anthocyanins in the red cabbage leaves and jabuticaba peels in dry solid base as a function of time and degradation temperature along with experimental uncertainties of TMA. Figure 1 shows the degradation profiles and Figure 2 shows the behavior of the first-order model. In general, as can be seen from Tables 1 and 2, the concentration of TMA statistically decreased with time at all studied temperatures. Moreover, the experimental behavior of TMA from red cabbage was sharper in relation to jabuticaba peels as the differences between means were observed at most of the evaluated data points. In what concerns the jabuticaba peels, the differences were more pronounced after 8 h of storage.

Table 1: Concentration of total monomeric anthocyanins (TMA) expressed as mg·g_{db}^{-1} in red cabbage leaves as a function of temperature and degradation time (t) along with experimental uncertainty.

***t* (h)**	**TMA 323.15 K**	**TMA 333.15 K**	**TMA 343.15 K**	**TMA 353.15 K**
0	1.394 ± 0.011^{aA}	1.445 ± 0.012^{aA}	1.349 ± 0.012^{aA}	1.321 ± 0.011^{aA}
10	1.244 ± 0.010^{bA}	1.145 ± 0.012^{bB}	0.839 ± 0.011^{bC}	0.701 ± 0.012^{bD}
20	1.166 ± 0.010^{cA}	0.903 ± 0.010^{cB}	0.584 ± 0.011^{cC}	0.331 ± 0.011^{cD}
30	1.086 ± 0.011^{dA}	0.629 ± 0.011^{dB}	0.410 ± 0.010^{dC}	0.168 ± 0.010^{cD}

Means with different small letters in the same column differ significantly ($p<0.05$). Means with different capital letters in the same row differ significantly ($p<0.05$).

Table 2: Concentration of total monomeric anthocyanins (TMA) expressed as mg·g_{db}^{-1} in jabuticaba peels as a function of temperature and degradation time (t) along with experimental uncertainty.

***t* (h)**	**TMA 323.15 K**	**TMA 333.15 K**	**TMA 343.15 K**	**TMA 353.15 K**	**TMA 363.15 K**
0	0.984 ± 0.010^{bA}	1.052 ± 0.010^{dA}	1.024 ± 0.011^{dA}	1.171 ± 0.012^{eB}	1.008 ± 0.011^{eA}
2	0.953 ± 0.011^{abA}	0.949 ± 0.012^{aA}	0.792 ± 0.010^{aB}	0.755 ± 0.011^{aB}	0.640 ± 0.010^{aC}
4	0.933 ± 0.011^{abcA}	0.901 ± 0.010^{aA}	0.745 ± 0.012^{aB}	0.645 ± 0.010^{bC}	0.313 ± 0.011^{bD}
6	0.904 ± 0.010^{acdE}	0.803 ± 0.010^{bA}	0.636 ± 0.010^{bB}	0.439 ± 0.010^{cC}	0.152 ± 0.010^{cD}
8	0.873 ± 0.010^{cdE}	0.782 ± 0.011^{bA}	0.557 ± 0.011^{cB}	0.372 ± 0.010^{cdC}	0.089 ± 0.011^{cdD}
10	0.853 ± 0.011^{dE}	0.625 ± 0.010^{cA}	0.511 ± 0.010^{cB}	0.303 ± 0.011^{dC}	0.046 ± 0.011^{dD}

Means with different small letters in the same column differ significantly ($p<0.05$). Means with different capital letters in the same row differ significantly ($p<0.05$).

Regarding the extraction, it should be noted that the process used allows for the extraction of a larger quantity of anthocyanins from red cabbage, with an average value of 1.3774 mg·g_{db}^{-1}, when compared to jabuticaba, with an average value of 1.0479 mg·g_{db}^{-1}, representing an increase of 31% in the extraction capacity. The observed difference in the yields of extraction can be attributed to many aspects. It can be cited the different type of anthocyanin present in the material and its specific

interaction with water. Moreover, the diffusion of water through the material depends on the characteristics of the plant tissue.

The results suggest that the anthocyanins in the red cabbage leaves, using water as solvent, are much more thermally stable than the anthocyanins in the jabuticaba peels. This finding is believed to be due to the effect of co-pigmentation that occurs in the red cabbage extracts [32]. For both evaluated materials, the study suggests a considerable stability until the temperature of 323.15 K. At the temperature of 333.15 K there is a much greater influence of the temperature on degradation. Studies in the literature [33 - 35] also suggest a more pronounced instability of anthocyanins with temperatures exceeding 333.15 K.

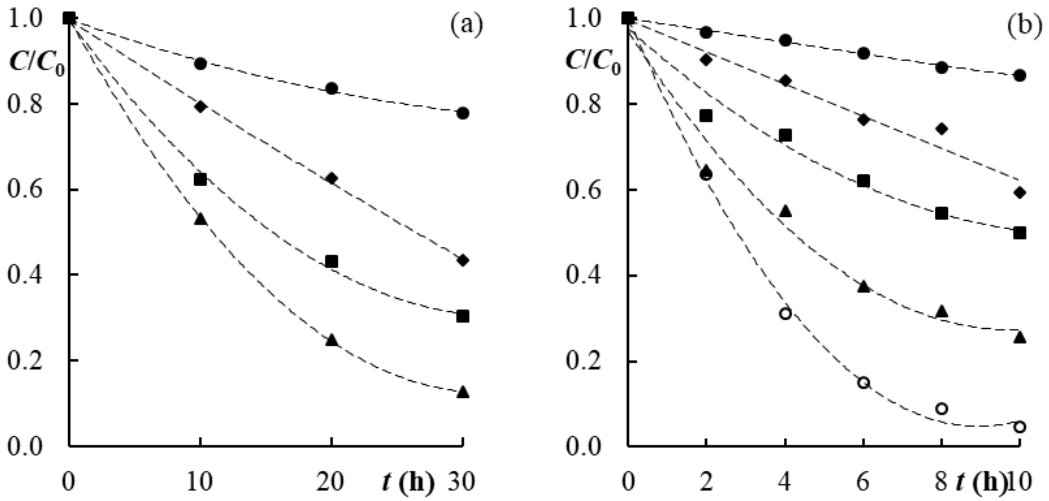

Figure 1: Degradation profile of anthocyanins in red cabbage (a) and jabuticaba (b): ● 323.15 K, ♦ 333.15 K, ■ 343.15 K, ▲ 353.15 K, ○ 363.15 K.

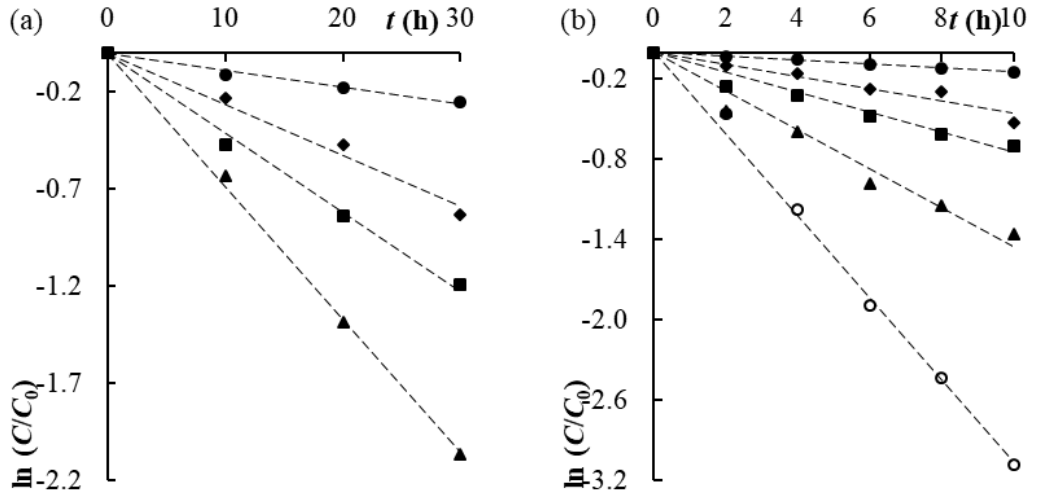

Figure 2: First-order model behavior for the degradation of the anthocyanins in red cabbage (a) and jabuticaba (b): ● 323.15 K, ♦ 333.15 K, ■ 343.15 K, ▲ 353.15 K, ○ 363.15 K.

In the evaluated time and temperature intervals, the degradation of anthocyanins in the red cabbage leaves and jabuticaba peels satisfactorily follow a first-order reaction, given that the worst correlation coefficient in the adjustment of the model to the experimental data was 0.9817 and 0.9433 for the cabbage and jabuticaba, respectively. The results for the activation energy reveal similar values for the degradation of anthocyanins in the two materials under study, 64.8 $kJ \cdot mol^{-1}$ for the red cabbage and 70.2 $kJ \cdot mol^{-1}$ for the jabuticaba, following the same order of magnitude as previously published studies [36 - 38].

The kinetic degradation parameters for both the red cabbage leaves and jabuticaba peels at each temperature under study are presented in Table 3. One can see that the values of the kinetic constant

k increase as the temperature increases, confirming the idea that the degradation of anthocyanins increases as the temperature increases. The $t_{1/2}$ values for the anthocyanins varied between 2.2 and 85.2 h, where the higher values indicate a greater thermal stability of the anthocyanins present in the red cabbage leaves.

The thermodynamic activation quantities, also shown in Table 3, reveal similar values for the degradation of anthocyanins in both the red cabbage and jabuticaba. The positive values of $\Delta H^{\ddagger}$ are associated with the need for energy in the breaking of the bonds while the reagents reach the activated state in an endothermic and non-spontaneous transformation, as suggested by the positive values of $\Delta G^{\ddagger}$.

Table 3: Kinetic constant (K), half-life time ($t_{1/2}$), Gibbs energy of activation ($\Delta G^{\ddagger}$), activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($T \cdot \Delta S^{\ddagger}$) for the degradation at different temperatures (T) of the anthocyanins in red cabbage leaves and jabuticaba leaves.

T (K)	K (h^{-1})	$t_{1/2}$ (h)	$\Delta G^{\ddagger}$ (kJ·mol^{-1})	$\Delta H^{\ddagger}$ (kJ·mol^{-1})	$T \cdot \Delta S^{\ddagger}$ (kJ·mol^{-1})
			Red cabbage		
323.15	0.0081	85.2	114.29	62.13	-52.16
333.15	0.0273	25.3	114.56	62.05	-52.51
343.15	0.0394	17.6	117.04	61.97	-55.07
353.15	0.0694	10.0	118.87	61.88	-56.98
			Jabuticaba		
323.15	0.0145	47.9	112.75	67.59	-45.16
333.15	0.0472	14.7	113.05	67.50	-45.54
343.15	0.0669	10.4	115.52	67.42	-48.10
353.15	0.1327	5.2	116.97	67.34	-49.63
363.15	0.3150	2.2	117.75	67.25	-50.50

The activation entropy is associated with the number of molecules that have enough energy to react [39], negative values of $\Delta S^{\ddagger}$ indicate that the molecules have less structural freedom than the reagents in the transition state so that a larger amount of energy is required for the formation of the activated complex.

4. CONCLUSION

The thermal degradation study of the anthocyanins in the red cabbage leaves and jabuticaba peels was successfully correlated by a first-order model. When comparing the degradation of the anthocyanins in the red cabbage with the degradation of the anthocyanins in the jabuticaba peels, one can see that the anthocyanins in the cabbage are less susceptible to thermal degradation. The evaluation of kinetic parameters generated by the transition-state theory suggests that the degradation of anthocyanins in the two evaluated materials is endothermic and not spontaneous. The thermodynamic parameters can help to understand the nature of the thermal degradation of anthocyanin extract from the two investigated species.

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