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# Thermostability of the visual color and anthocyanins from Rio-Grande-Cherry (*Eugenia involucrata* DC)

## *Termoestabilidade da cor e das antocianinas de Cerejeira-do-Rio-Grande (Eugenia involucrata DC)*

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## Abstract

The extract of Rio-Grande-Cherry (*Eugenia involucrata* DC), pure and with stevia addition (0.75% and 1.5%) and sucrose addition (20% and 40%), was subjected to heat treatment at 10 °C, 25 °C and between 50 °C and 90 °C. Anthocyanins and the color parameters C* and TCD (total color difference) followed first-order reaction kinetics while h° followed a zero order kinetic model, under all conditions. The addition of sweeteners, through the reduction of water activity, influenced the thermal stability of the anthocyanins and of the color parameters C*, h° and TCD was the most pronounced effect in the latter. The lower the temperatures, the most relevant was the effect of the sweeteners on increasing half-life. Thermodynamically, the degradation reaction of anthocyanins was defined as endothermic, as well as non-spontaneous and transition state of the molecules more structurally organized than the reactants.

**Keywords:** Degradation; Pigment; Kinetic; Stevia; Sucrose; TCD; Enthalpy.

## Resumo

O extrato de antocianinas de Cerejeira-do-Rio-Grande (*Eugenia involucrata* DC), puro e adicionado de stevia (0,75% and 1,5%) e sacarose (20% e 40%), foi submetido a tratamento térmico a 10 °C, 25 °C e entre 50 °C e 90 °C. As antocininas e os parâmetros C* e TCD (Diferença Total de Cor) da cor seguiram um modelo de cinética de primeira ordem enquanto o parâmetro h° seguiu ordem zero, em todas as condições. A adição dos adoçantes, através da redução da atividade de água, influenciou a estabilidade térmica das antocininas e dos parâmetros da cor C*, h° e TCD sendo o efeito mais pronunciado neste último. Quanto mais baixas as temperaturas, mais acentuado foi o efeito dos adoçantes no aumento da meia-vida. Termodinamicamente, a reação de degradação das antocianinas foi caracterizada como endotérmica, não espontânea e com o estado de gtransição das moléculas mais organizado estruturalmente do que os reagentes.

**Palavras-chave:** Degradação; Pigmento; Cinética; Stevia; Sacarose; Diferença Total de Cor; Entalpia.

## 1 Introduction

Anthocyanins are a subgroup within the family of flavonoids and are the largest group of water-soluble pigments in plants. They are highly appreciated in the food industry for their colouring properties, which can give foods salmon-pink, orange, red, violet and blue colors (Sharma et al., 2016). As it is still a sources rich in natural antioxidants (Lee et al., 2013), its beneficial effects on health make it a potential natural substitute for artificial colors, especially for foods.

Rio-Grande-Cherry (*Eugenia involucrata* DC) is a native species from the south of Brazil, rich in anthocyanisn that can be found from Minas Gerais to Rio Grande do Sul states, whose fruits ripen from October to December (Cripa et al., 2014). Its fruit is appreciated in natura and can also be processed for the production of candies and jellies by the family agribusiness (Golle et al., 2012). Cyanidin-3-glucoside are major anthocyanins in *E. involucrata* DC and these anthocyanins are liable compounds and easily susceptible to degradation through factors such as light, pH, and temperature during storage and especially heat processing (Fazaeli et al., 2013; Tsai et al., 2014). Many food items are thermally processed prior to consumption and this process may greatly influence the anthocyanin content and species as well as the biological activity of the final product (Zhou et al., 2018). When color is a significant sensory property in determining product quality, minimizing pigment loss during processing is the main concern of the processor (Markakis, 1982). Accordind to Pérez-Ramírez et al. (2015), recently, the effect of additives, such sweeteners, have been evaluated in order to preserve the color of the extract. These additives has been shown to be a viable alternative that, in addition to low cost, is an ingredient present in candies, sweets and other foods. According to Ertan et al. (2019), high sugar concentration can stabilize anthocyanins by reducing water activity.

Thus, the determination of kinetic and thermodynamic parameters is essential to predict the possible quality changes that occur during thermal processing (Kara & Erçelebi, 2013). Therefore, the objective of this work was to characterize kinetic and thermodynamically the degradation of visual color and anthocyanins extracted from Rio-Grande-Cherry at 10, 25, 50, 60, 70, 80 and 90 °C with stevia and sucrose addition.

## 2 Method

### 2.1 Pigment extraction

Rio-Grande-Cherry were collected in the city of Santo Antônio da Patrulha, RS, Brazil and stored in plastic pots and frozen at an average temperature of -11 °C in the Laboratory of Thermodynamics and Kinetics of Degrading Processes (LTCPD-FURG). The seedless fruits were mechanically ground in a mixer and anthocyanins were extracted according to Favaro (2008), using ethanol 99% as the solvent (ratio 1:3 w/v), at 55 °C, during 30 minutes. After extraction, the extract was vacuum filtered and stored refrigerated (5.0 ± 1.0 °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. The pH was adjusted to 3 with HCl (0.1%) to ensure the stability of the anthocyanins.

## 2.2 Anthocyanins concentration

The total content of anthocyanins was calculated according to Fuleki & Francis (1968), as described in Equation 1.

$$C_A = \left( \frac{Abs\,x\,MM\,x\,FD}{\varepsilon} \right) x\,1000 \tag{1}$$

where $C_A$ is the concentration of total anthocyanins (mg of anthocyanins 100 g of sample$^{-1}$), Abs is the absorbance of the extract read at 535 nm, PM is the molar mass (664.5 g mol$^{-1}$) of cyanidin-3.5-glycoside, FD is the dilution factor and ε is the molar extinction coefficient of anthocyanin (1.25x$10^3$ L mol$^{-1}$ cm$^{-1}$) at the established wavelength.

## 2.3 Visual color measurements

The color was evaluated using the colorimeter (Minolta®, model CR400) and the color parameters were expressed with L* (brightness), a* (red/green) and b* (yellow/blue). According to Konica Minolta (1993) the chroma value (C*), Hue angle (h°) and the total color difference (TCD) are calculated through Equations 2, 3 and 4.

$$C^* = \left[ \left(a^*\right)^2 + \left(b^*\right)^2 \right] / 2 \tag{2}$$

$$h^0 = tan^{-1}\left(b^* / a^*\right) \tag{3}$$

$$TCD = \sqrt{\left(L_0 * - L *\right)^2 + \left(a_0 * - a *\right)^2 + \left(b_0 * - b *\right)^2} \tag{4}$$

where $L_o$*, $a_o$* and $b_o$* are the initial color readings, with no heat treatment, and L*, a* and b* are the color parameter readings at a given time and temperature.

## 2.4 Thermal degradation

Aliquots of 5 mL of anthocyanins diluted extract (1:1) from Rio-Grande-Cherry were placed in screw cap test tubes and subjected to heat treatment at temperatures between 50 °C and 90 °C, in thermostatic water batch. At 25° C, test tubes were kept on a bench and at 10 °C in refrigeration and; in all conditions the test tubes were protected from light. In a regular interval of time the samples of the pure extract (control) as well as those of 0.75% and 1.5% (m/v) of stevia (ST) and 20% and 40% (m/v) of sucrose (SUC) (Rubinskiene et al., 2005; Pérez-Ramírez et al., 2015) were removed to ensure that half the initial anthocyanin concentration was reached. Samples submitted to the thermostatic water bath at 50, 60, 70, 80 and 90 °C were taken every 180, 60, 30, 15 and 10 min. Once removed, the samples were subjected to spectrophotometric reading to determine the anthocyanin concentration and the color parameters were measured. At 10 and 25 °C, samples were taken at 3-day intervals.

Degradation of the extracts was monitored by spectrophotometric measurements at 535 nm, and all assays were performed in triplicate. This wavelength value corresponds to the maximum absorbance in the region between 400 and 600 nm, where the absorption of the flavil cation occurs, one of the forms of anthocyanin present in Rio-Grande-Cherry and very stable in acidic medium.

## 2.5 Determination of kinetic and thermodynamic parameters

The degradation rate constant of the anthocyanin extract $k_d$ ($h^{-1}$), C* and TCD were estimated according to Equation 5 (Fogler, 2012), assuming first-order reaction kinetics (Kara & Erçelebi, 2013):

$$\frac{dC_A}{dt} = -k_d \, x \, C_A \tag{5}$$

The changes in Hunter color values h° were modeled according to zero-order kinetics model, according to Equation 6 (Fogler, 2012):

$$\frac{dC_P}{dt} = k_d \tag{6}$$

where $C_P$ is the colorimetric parameter h° at a given time and t is the time (h).

The half-life (h) of the extract is given by Equation 7 (Fogler, 2012):

$$t_{1/2} = \ln(2)/k_d \tag{7}$$

Dependence of the degradation rate constant on temperature is represented by the Arrhenius Equation 8 (Fogler 2012):

$$\ln k_d = \ln A = Ea/(R\,x\,T) \tag{8}$$

where Ea is the activation energy (kJ $mol^{-1}$), A is the frequency factor ($h^{-1}$), T is the temperature (K) and R is the universal gas constant (0.0083 kJ $gmol^{-1}$ $K^{-1}$).

The activation enthalpy (ΔH) and entropy (ΔS) as well as the inactivation free Gibbs energie (ΔG) were determined according to Equations 9, 10 and 11 (Smith et al., 2007):

$$\Delta H = Ea - (R\,x\,T) \tag{9}$$

$$\Delta G = R\,x\,T\,x\,\ln\left[(k_d\,x\,h)/(k_b\,x\,T)\right] \tag{10}$$

$$\Delta S = (\Delta H - \Delta G)/T \tag{11}$$

where h is Planck's constant ($6.6262 \times 10^{-34}$ J s) and $k_b$ is Boltzmann's constant ($1.3806 \times 10^{-23}$ J $K^{-1}$).

## 2.6 Statistical analysis

The results were treated by analysis of variance, followed by the Tukey test, considering a confidence level of 95% ($p < 0.05$).

## 3 Results and discussion

### 3.1 Thermal degradation kinetics of anthocyanins

The degradation of anthocyanins from *E. involucrata* between 10 and 90 °C followed first-order reaction kinetics as described by Karasu et al. (2016) and Loypimai et al. (2016). For all analyzed conditions of the extract (Table 1) there was an increase of $k_d$ as well as a reduction in $t_{1/2}$ when temperature was increased from 10 °C to 90 °C, as expected. Mercali et al. (2013) and Peron et al. (2017) reported the same behavior for the anthocyanins extracted from acerola pulp with water at temperatures between 75 °C and 90 °C, and anthocyanins extracted from Juçara with ethanol and water, between 50 °C and 90 °C, respectively.

High temperatures cause the formation of chalcones as a consequence of opening the pyrilium ring in the anthocyanin molecule. The latter would be cleaved to produce chalcone, which, due to its thermolability, degrades instantly into a phenolic acid and an aldehyde. As it has a larger group of hydroxyls, cyanidins are able to release a greater amount of H + protons in the reaction medium. Thus, equilibrium will shift in the direction of of flavilium cation formation, influencing the rate of degradation reaction (Cabrita et al., 2014).

According to published results from previous studies, the influence of sugars on the anthocyanins presents controversial results in relation to the stability or instability provided. Kopjar et al. (2012) described that influence in relation to the addition of trehalose (10%) or glucose (10%) in blackberry juices, which significantly reduced anthocyanin degradation during storage, whereas the opposite effect was observed when 10% of fructose or sucrose was incorporated.

In a high water activity range, the reagents are in solution and this dilution reduces the reaction rate through the Law of Mass Action. Thus, the reaction rate is proportional to the concentration of the reactant, which decreases with increasing water content (Karel & Lund, 2003; Jiménez et al., 2012).

At the temperature of 90 °C, the addition of 1.5% of stevia to the extract increased half-life in 13.2% (and reduced $k_d$) over that obtained for the control sample. Pérez-Ramírez et al. (2015) explained that with stevia addition there is a decrease in the formation of chalcones of brown color during the conditions of storage in low temperatures, due to a reduction in water activity, which shifts the dehydration equilibrium relative to the flavilium cation by the removal of water, thus retarding its rate of hydration.

Between 60 °C and 80 °C, the addition of the sweeteners did not promote a gain in the half-life of the extract. However, at 50 °C and 25 °C, the addition of 40% sucrose increased the half-life of the extract in 12.7% and 31.1%, in this order. At 10 °C, 20% sucrose increased half-life in 147% while stevia in the two concentrations and 40% sucrose increased 104.6%on average, as expected, considering that at lower temperature, anthocyanins are more stable, which contributes to the increment determined with the incorporation of the sweeteners.

**Table 1.** Effects of stevia (ST) and sucrose (SUC) in the $k_d$ and $t_{1/2}$ values on thermal degradation of anthocyanins.

| | 90 °C | | 80 °C | | 70 °C | | 60 °C | | 50 °C | | 25 °C | | 10 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) |
| Control | 0.180[a] (0.90) | 3.8 | 0.108[a] (0.95) | 6.4 | 0.072[a] (0.96) | 9.6 | 0.036[a] (0.95) | 19.2 | 0.018[cd] (0.96) | 38.5 | 0.003[a] (0.96) | 231 | 0.00306[a] (0.87) | 226.5 |

| | 90 °C | | 80 °C | | 70 °C | | 60 °C | | 50 °C | | 25 °C | | 10 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ST 0.75% | 0.180[a] (0.90) | 3.8 | 0.108[a] (0.95) | 6.4 | 0.108[b] (0.97) | 6.4 | 0.072[b] (0.97) | 9.6 | 0.025[ab] (0.92) | 27.7 | 0.003[a] (0.96) | 231 | 0.00162[b] (0.88) | 427.9 |
| ST 1.5% | 0.160[c] (0.90) | 4.3 | 0.108[a] (0.97) | 6.4 | 0.108[b] (0.97) | 6.4 | 0.036[a] (0.95) | 19.2 | 0.025[ab] (0.93) | 27.7 | 0.0027[ab] (0.96) | 256.7 | 0.00144[b] (0.92) | 481.3 |
| SUC 20% | 0.180[a] (0.95) | 3.8 | 0.108[a] (0.97) | 6.4 | 0.072[a] (0.94) | 9.6 | 0.036[a] (0.97) | 19.2 | 0.025[ab] (0.95) | 27.7 | 0.0025[ab] (0.96) | 277.2 | 0.00126[c] (0.91) | 550.1 |
| SUC 40% | 0.324[b] (0.97) | 2.1 | 0.180[bc] (0.99) | 3.8 | 0.108[b] (0.99) | 6.4 | 0.036[a] (0.96) | 19.2 | 0.016[d] (0.92) | 43.3 | 0.0023[b] (0.96) | 301.4 | 0.00144[b] (0.96) | 481.3 |

[1]Equal letters indicate that there is no statistical difference between the means in each column ($p < 0.05$); [2]Coefficients of determination ($R^2$) shown in parentheses.

To Tonon et al. (2010), the higher the water content, the higher is the molecular mobility inside the food, which facilitates the physicochemical reactions of degradation. According to Chu et al. (2016), the addition of sucrose exerted a protective effect under that pigment, inhibiting the degradation of anthocyanins of *Clitoria ternatea* extract at 60 °C.

Heldman (2011) determined that the activation energies for the degradation of anthocyanins vary from 35 to 125 kJ $mol^{-1}$ and the data obtained are within this range, 47.36 (0.96) for pure extract and 54.23 (0.97), 54.3 (0.98), 54.3 (0.99) and 61.15 (0.98) kJ $mol^{-1}$, with 0.75 and 1.5% of stevia, with 20% and 40% of sucrose, respectively.

The addition of sweeteners in anthocyanins extract from Rio-Grande-Cherry increased the activation energy, which indicates a strong temperature dependence, which means that the reaction occurs more slowly at low temperature and is relatively faster at high temperatures. However, anthocyanin extract from Rio-Grande-Cherry was less affected by temperature and therefore, degrades more slowly than other sources, such as acerola. For the degradation reaction of anthocyanins in the pulp of this fruit, Mercali et al. (2013) determined an activation energy equal to 74.8 kJ $mol^{-1}$ between 75 and 90 °C.

According to Georgieva et al. (2012), activation enthalpy ($\Delta H$) shows the energy difference between the reagent and activated complex. For the three analyzed conditions of the extract (pure, with stevia addition and with sucrose addition), the temperature increase maintained the values of $\Delta H$ (Table 2).

Similar values indicate that the energy barrier that must be overcome in order to achieve the transition state is similar, and the positive sign means that anthocyanin degradation is an endothermic reaction, accompanied by the adsorption of heat. Mercali et al. (2013) and Peron et al. (2017) obtained the same behaviour when they studied degradation of acerola pulp and "Italia" grape (*Vitis vinifera* L. Brasil) extract, respectively.

Considering the free energy of inactivation ($\Delta G$), which represents the difference between the activated state and the state of the reactants, the positive values indicated that anthocyanin degradation is a non-spontaneous reaction and the similar values indicated similar degradation mechanisms under the different conditions for the same temperature, as observed by Izaguirres et al. (2018) when analyzing the influence of the addition of sucrose and fructose on the thermal degradation of betanins. The activation entropy ($\Delta S$) represents the change in disorder of molecules in the system and the negative values obtained suggest that the transition state is more organized than the reactants, and therefore, the formation of the activated complex is associated with a decrease of entropy (Mercali et al., 2015).

**Table 2.** Thermodynamic parameters obtained for thermal degradation of anthocyanin with stevia (ST) and sucrose (SUC) addition.

| Thermodynamic parameters | | 90 °C | 80 °C | 70 °C | 60 °C | 50 °C |
|---|---|---|---|---|---|---|
| ΔH (kJ $gmol^{-1}$) | Control | 44.13 | 44.22 | 44.30 | 44.38 | 44.47 |
| | ST 0.75% | 51.00 | 51.09 | 51.17 | 51.25 | 51.34 |
| | ST 1.5% | 51.07 | 51.16 | 51.24 | 51.32 | 51.41 |
| | SUC 20% | 51.07 | 51.16 | 51.24 | 51.32 | 51.41 |
| | SUC 40% | 57.92 | 58.01 | 58.09 | 58.17 | 58.26 |
| ΔG (kJ $gmol^{-1}$) | Control | 127.82 | 126.05 | 123.87 | 122.49 | 120.99 |
| | ST 0.75% | 127.82 | 126.05 | 122.63 | 120.43 | 120.04 |
| | ST 1.5% | 128.20 | 126.05 | 122.63 | 122.49 | 120.04 |
| | SUC 20% | 127.82 | 126.05 | 123.87 | 122.49 | 120.04 |
| | SUC 40% | 125.93 | 124.45 | 122.63 | 122.49 | 121.33 |
| -ΔS (kJ $gmol^{-1}K^{-1}$) | Control | 215.69 | 216.49 | 216.23 | 218.17 | 219.90 |
| | ST 0.75% | 197.98 | 198,32 | 194.19 | 193.22 | 197.43 |
| | ST 1.5% | 198.78 | 198.13 | 194.00 | 198.79 | 197.22 |
| | SUC 20% | 197.80 | 198.13 | 197.37 | 198.79 | 197.22 |
| | SUC 40% | 175.26 | 175.76 | 175.39 | 179.65 | 181.12 |

ΔH = activation enthalpy. ΔG = free energy of inactivation. ΔS = activation entropy.

As ΔH, for the pure extract, stevia addition and sucrose addition, the temperature increase maintained the values of ΔS. Thermodynamically, the incorporation of 40% sucrose was more promising in increasing the stability of the anthocyanin extract, since the ΔS values were lower and ΔH values were higher than those obtained with stevia or 20% sucrose, for all temperatures. According Georgieva et al. (2012), in relation to activation entropy, this means that the anthocyanins has just passed through some kind of physical or chemical rearrangement of the initial structure, bringing it to a state close to its own thermodynamic equilibrium, which indicates that it is little reactive

### 3.2 Thermal degradation kinetics of visual color

According to Table 3, the data adjustments ($R^2$) confirmed zero-order kinetics model for h° and a first-order kinetic model for C* and TCD parameters as Yang et al. (2008) determined. Figure 1 shows the degradation behavior at 90 °C of h° and C* parameters of the extract with 1.5% and 0.75% stevia, in this order..

In a zero-order reaction, the rate of conversion of the reactant depends only on the time, whereas in a first-order reaction the rate also depends on the concentration of the reactants involved.

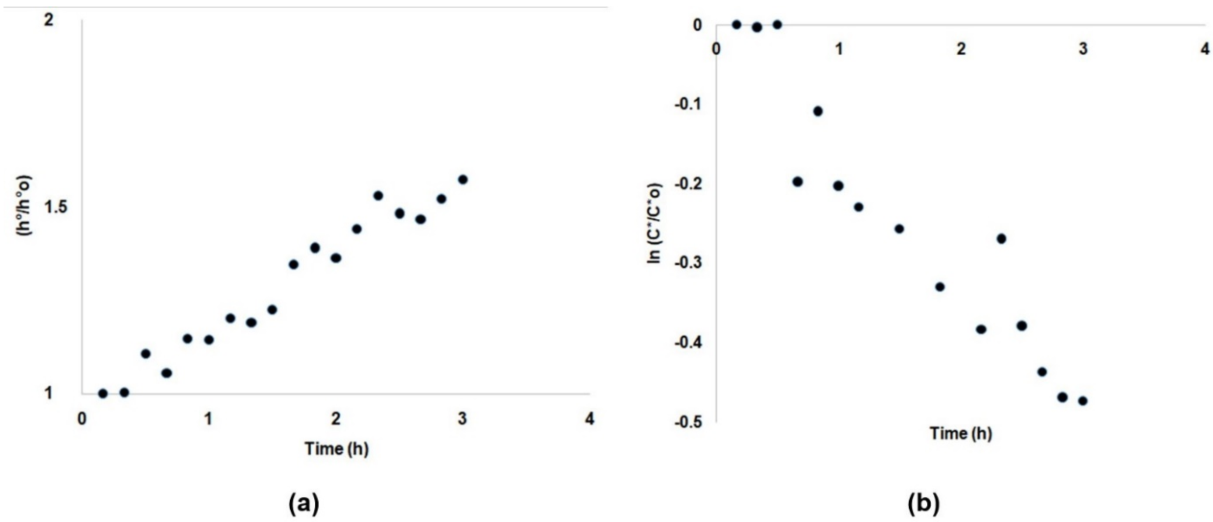


**Figure 1.** Degradation of h° (a) and C* (b) parameters of Rio-Grande-Cherry extract with stevia 1.5% ($R^2 = 0.96$) and stevia 0.75% ($R^2 = 0.94$) during heating at 90 °C, respectively.

**Table 3.** Effects of stevia (ST) and sucrose (SUC) in the $k_d$ values on visual color parameters.

| Visual color parameteres | | 90 °C | | 80 °C | | 70 °C | | 60 °C | | 50 °C | | 25 °C | | 10 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) | $k_d$ ($h^{-1}$)[1,2] | $t_{1/2}$ (h) |
| C* | Control | $0.208^{a}$ (0.91) | 3.3 | $0.167^{a}$ (0.84) | 4.1 | $0.068^{a}$ (0.92) | 10.2 | $0.088^{a}$ (0.96) | 7.9 | $0.015^{a}$ (0.90) | 46.2 | $0.004^{a}$ (0.90) | 173.3 | $0.0024^{a}$ (0.93) | 288.8 |
| | ST 0.75% | $0.196^{a}$ (0.9) | 3.5 | $0.064^{b}$ (0.58) | 10.8 | $0.071^{a}$ (0.95) | 9.8 | $0.091^{a}$ (0.98) | 7.6 | $0.019^{a}$ (0.90) | 36.5 | $0.007^{b}$ (0.91) | 99.0 | $0.0023^{a}$ (0.93) | 301.4 |
| | ST 1.5% | $0.222^{a}$ (0.91) | 3.1 | $0.117^{a}$ (0.91) | 5.9 | $0.072^{a}$ (0.97) | 9.6 | $0.098^{a}$ (0.98) | 7.1 | $0.015^{a}$ (0.91) | 46.2 | $0.008^{b}$ (0.91) | 86.6 | $0.0024^{a}$ (0.78) | 288.8 |
| | SUC 20% | $0.229^{a}$ (0.9) | 3.0 | $0.132^{a}$ (063) | 5.2 | $0.075^{a}$ (0.95) | 9.2 | $0.088^{a}$ (0.97) | 7.9 | $0.017^{a}$ (0.95) | 40.8 | $0.004^{a}$ (0.90) | 173.3 | $0.0015^{b}$ (0.94) | 462.1 |
| | SUC 40% | $0.181^{a}$ (0.92) | 3.8 | $0.081^{c}$ (0.92) | 8.5 | $0.075^{a}$ (0.92) | 9.2 | $0.073^{b}$ (0.95) | 9.5 | $0.016^{a}$ (0.96) | 43.3 | $0.001^{c}$ (0.87) | 693.1 | $0.0013^{bc}$ (0.92) | 533.2 |
| h° | Control | $0.117^{a}$ (0.91) | 5.9 | $0.060^{a}$ (0.76) | 11.5 | $0.038^{a}$ (0.98) | 18.2 | $0.007^{a}$ (0.92) | 99.0 | $0.008^{a}$ (0.97) | 86.6 | $0.002^{a}$ (0.90) | 346.6 | $0.0019^{a}$ (0.95) | 364.8 |
| | ST 0.75% | $0.121^{a}$ (0.95) | 5.7 | $0.059^{a}$ (0.78) | 11.7 | $0.037^{a}$ (0.97) | 18.7 | $0.007^{a}$ (0.89) | 99.0 | $0.008^{a}$ (0.96) | 86.6 | $0.002^{a}$ (0.81) | 346.6 | $0.0016^{a}$ (0.94) | 433.2 |
| | ST 1.5% | $0.136^{a}$ (0.96) | 5.1 | $0.141^{b}$ (0.69) | 4.9 | $0.040^{a}$ (0.98) | 17.3 | $0.007^{a}$ (0.92) | 99.0 | $0.009^{a}$ (0.94) | 77.0 | $0.003^{a}$ (0.81) | 231.0 | $0.0018^{a}$ (0.97) | 385.1 |
| | SUC 20% | $0.142^{a}$ (0.93) | 4.9 | $0.086^{c}$ (0.86) | 8.0 | $0.040^{a}$ (0.96) | 17.3 | $0.007^{a}$ (0.93) | 99.0 | $0.008^{a}$ (0.97) | 86.6 | $0.002^{a}$ (0.79) | 346.6 | $0.0019^{a}$ (0.94) | 364.8 |
| | SUC 40% | $0.147^{a}$ (0.93) | 4.7 | $0.061^{a}$ (0.52) | 11.4 | $0.045^{a}$ (0.97) | 15.4 | $0.003^{b}$ (0.90) | 231.0 | $0.008^{a}$ (0.97) | 86.6 | $0.001^{b}$ (0.87) | 693.1 | $0.0021^{b}$ (0.92) | 330.1 |
| TCD | Control | $0.117^{a}$ (0.92) | 5.9 | $0.051^{a}$ (0.92) | 13.6 | $0.106^{a}$ (0.93) | 6.5 | $0.043^{a}$ (0.92) | 16.1 | $0.021^{a}$ (0.94) | 33.0 | $0.004^{a}$ (0.91) | 173.3 | $0.0037^{a}$ (0.94) | 187.3 |
| | ST 0.75% | $0.112^{a}$ (0.90) | 6.2 | $0.038^{b}$ (0.91) | 18.2 | $0.112^{a}$ (0.95) | 6.2 | $0.051^{a}$ (0.91) | 13.6 | $0.020^{a}$ (0.91) | 34.6 | $0.005^{a}$ (0.89) | 138.6 | $0.0031^{b}$ (0.92) | 223.6 |
| | ST 1.5% | $0.098^{a}$ (0.90) | 7.1 | $0.043^{b}$ (0.90) | 16.1 | $0.128^{a}$ (0.92) | 5.4 | $0.037^{a}$ (0.95) | 18.7 | $0.020^{a}$ (0.92) | 34.6 | $0.003^{b}$ (0.96) | 231.0 | $0.0020^{c}$ (0.90) | 346.6 |
| | SUC 20% | $0.111^{a}$ (0.92) | 6.2 | $0.034^{b}$ (0.91) | 20.4 | $0.170^{a}$ (0.94) | 4.1 | $0.050^{a}$ (0.92) | 13.9 | $0.018^{a}$ (0.92) | 38.5 | $0.002^{c}$ (0.90) | 346.6 | $0.0017^{c}$ (0.92) | 407.7 |
| | SUC 40% | $0.113^{a}$ (0.91) | 6.1 | $0.062^{a}$ (0.88) | 11.2 | $0.134^{a}$ (0.93) | 5.2 | $0.051^{a}$ (0.90) | 13.6 | $0.013^{c}$ (0.82) | 53.3 | $0.002^{c}$ (0.90) | 346.6 | $0.0015^{c}$ (0.90) | 462.1 |

[1]Equal letters indicate that there is no statistical difference between the means in each column ($p < 0.05$). [2]Determination coefficients ($R^2$) shown in parentheses.

With increasing temperature, the increase of the values of $k_d$ of the parameters C* and h° indicated the reduction of the chroma and the decrease in red staining. This demonstrated the decrease of the red coloration (h° = 0) that can be associated with the formation of yellow chalcones (h° = 90), while that same increase over the TCD parameter represented significant losses of the color of the original sample.

The TCD parameter was the most affected by the presence of sweeteners, since in, at least, two temperatures for each of the four conditions proposed, there was an increase in half-life and consequent decrease in the total color degradation process.

At lower temperature, 10 °C, stevia and sucrose at both concentrations reduced the $k_d$ values while the addition of 1.5% of stevia and 20% of sucrose was effective at 10 °C, 25 °C and 80 °C. With 40% of sucrose added, the degradation of TCD was less pronounced at 10 °C, 25 °C and 50 °C. Yet, the behavior of the TCD parameter between 10 and 90 °C suggested that the total color difference degradation may reflect anthocyanin degradation during thermal processing. The presence of 40% of sucrose in the extract allowed a reduction in the degradation process of the parameters C* at 10 °C, 25 °C, 60 °C and 80 °C and h° at 25 °C and 60 °C.

When Yang et al. (2008) studied the thermal degradation kinetics of anthocyanins from purple corn (*Zea mays* L.) between 70 °C and 90 °C, the values of activation energies (Ea) of the visual color degradation reaction with respect to the parameters C*, h° and TCD were compatible with expected color changes for food products, which according to Lund (1977) should be in the range of 40 to 125 kJ $gmol^{-1}$. The Ea values obtained for C* were 45.22 for pure extract and 46.11, 46.81, 58.62 and 51.44 kJ $mol^{-1}$, with 0,75% and 1,5% of stevia, with 20% and 40% of sucrose, respectively. The h° values were 48.44, 50.7, 62.58, 55.03 and 53.91 and to TCD were 27.38, 38.84, 40.14, 52.28 and 52.48 kJ $mol^{-1}$, in that order. Also, they showed the same degradative behavior of the anthocyanin: the addition of sweeteners increased the activation energy in relation to the pure extract (control).

## 4 Conclusions

To elucidate the behavior of anthocyanins and visual color parameters under heating, it is possible toincrease the popularity of Rio-Grande-Cherry as a source of this natural pigment. The addition of sweeteners can reduce the thermal degradation of anthocyanins and color parameters, especially at lower temperatures. Although stevia and sucrose contributed to the increased stability of the extract from Rio-Grande-Cherry at both concentrations under certain experimental conditions, the incorporation of 40% sucrose stood out as the most effective in relation to all evaluated parameters, within the studied temperature range.

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