EFFECTS OF THE ADDITION OF SUGARS ON THE THERMOSTABILITY OF BETANIN FROM BETA VULGARIS L.

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O impacto da adição de açúcares na estabilidade das betaninas de *Beta vulgaris* L. (beterraba) durante o tratamento térmico entre 50 e 80°C e pH 5 foi investigado. A degradação das betaninas pura e adicionada de 10% de sacarose e 40% de frutose seguiu modelo de cinética de primeira ordem e conforme aumento da temperatura, houve aumento dos valores da constante de degradação térmica (Kd), redução na meia-vida e nos valores do tempo de redução decimal (D). Termodinamicamente, apresentando valores de variação de entalpia (ΔH) mais altos (entre 99,66 e 99,41 kJ mol^{-1}) que o extrato com a adição de sacarose (entre 85,27 e 85,03 kJ mol^{-1}) e controle (entre 78,85 e 78,61 kJ mol^{-1}), a incorporação de frutose foi mais promissora no aumento da estabilidade do extrato de betaninas. Os valores positivos para a variação da energia livre de Gibbs (ΔG) indicaram que a reação de degradação das betaninas foi não-espontânea e os baixos valores da variação de entropia (ΔS) para o extrato com frutose (entre 0,02 e 0,03 J mol^{-1}K^{-1}) indicaram que o estado do material estava próximo ao seu equilíbrio termodinâmico.

PALAVRAS-CHAVE: ESTABILIDADE; TERMODINÂMICA; SACAROSE; FRUTOSE; PIGMENTOS NATURAIS

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1 INTRODUCTION

Color provides the basic quality information for human perception, and directly influences consumer's acceptance and preference. It is the first quality attribute of food evaluated by consumers, and is therefore an important component of food quality relevant to market acceptance (Wu & Sun, 2013). Minimizing loss of pigment during processing is a primary concern for the processing industry — continuous monitoring of color change during industrial processing is necessary to ensure product quality (Shao-qian et al., 2011).

Mathematical modelling of experimental data can accurately predict the progress of a chemical reaction taking place in a homogeneous liquid or semi-solid during thermal processing or storage, being very useful in many engineering applications, including process optimization. So, experimental studies and applications of various simplified models to predict and interpret kinetics parameters (reaction order, rate constant, activation energy) are needed (Figueira et al., 2011). The important value of kinetic models is to predict quality changes inside and outside the test temperature range and optimize thermal processes under given non-isothermal conditions (Lixia et al., 2015). Most food quality attributes changes during thermal process or during storage follow a first order reaction model (Figueira et al., 2011). Topuz (2008), Patras et al. (2010) and Peron et al. (2017) have all considered the degradation of color to follow first-order reaction kinetics.

Motivated by the current global trend of using natural pigments as colorants for food, a search for economically viable sources has been conducted (Rossi et al., 2011). In this context, betalains are natural pigments typically associated with plants of the order *Cariofilales* and the major commercially exploited betalain crop is red beet (*Beta vulgaris* L.) (Vulíc et al., 2013). They include two classes of pigments: red betacyanins and yellow betaxanthins (Castellar et al., 2003). The most common betacyanin is betanidin 5-O-β-glucoside (betanin), the major pigment in red beets (Azeredo, 2009).

In addition to their red color, betalains possess several desirable biological activities, including antioxidant, antiinflammatory, hepatoprotective, and antitumor properties (Georgiev et al., 2010). However, it is known that temperature has a strong influence on the stability of betanin and the half-life of heat decomposition of betanin depends on the pH value, oxygen and light. Although the stability of betanin is also influenced by the water activity value, the addition of sugars may provide a protective effect against pigment decomposition (Stintzing & Carle, 2008; Kopjar et al., 2012).

The aim of this research was to evaluate the effect of sucrose and fructose incorporation during the thermal processing of betanin from *Beta vulgaris* L. in order to estimate the degradation kinetics parameters and the thermodynamic functions of activation.

2 MATERIALS AND METHODS

2.1 PIGMENT EXTRACTION

Samples of *Beta vulgaris* L. were collected in the city of Santo Antônio da Patrulha - RS and betanin was extracted according to Drunkler et al. (2003), using 70% ethanol as the solvent (ratio 1:2 w/v). 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.

2.2 THERMAL DEGRADATION

For processing between 50 and 80°C, 0.5 mL samples of the betanin extract added by 40% (m v^{-1}) of sucrose and 10% (m v^{-1}) of fructose (Rubinskiene et al., 2005) 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 betanin concentration was reached for each temperature condition explored. The pH of the extract was adjusted to 5.0 by the addition of 1 M HCl (Kasvi pH meter K39-2014B) to ensure the stability of the betanins (Castellar et al., 2003)

The initial betanin concentration (C_{BO}) and the concentration immediately after sample collection (C_B) were determined for each temperature and the results were expressed as C_{BO}/C_B.

2.3 BETANIN CONCENTRATION

The betanin concentration (C_B) was monitored spectrophotometrically (Bel Photonics Spectrophotometer SP 1105) using the absorbance at 538 nm and 600 nm according to Nilsson (1970) and was defined as (*Equation 1*):

$$C_B = \frac{[OD_{538} - OD_{600}] * DF}{1023} \tag{1}$$

where C_B is the betanin concentration (mg 100 g red beet^{-1}), OD_{538} is the optical density of the sample at 538 nm, OD_{600} is the optical density of the sample at 600 nm and DF is the dilution factor.

2.4 DEGRADATION RATE CONSTANT

The degradation rate constant of the betanin extract and K_d (h^{-1}) were estimated according to *Equation 2* (Fogler, 2012), assuming first order reaction kinetics:

$$\frac{dC_B}{dt} = -K_d C_B \tag{2}$$

where C_B is the betanin concentration (mg 100 g red beet^{-1}) and t is the time (h).

2.5 HALF-LIFE VALUES

The half-life $t_{1/2}$ (h) for the first-order degradation kinetic model is given by *Equation 3* (Fogler, 2012):

$$t_{1/2} = \frac{\ln 2}{K_d} \tag{3}$$

where K_d is the degradation rate constant (h^{-1}).

2.6. DECIMAL REDUCTION TIME

The decimal reduction time D (h) which is the time needed for a tenfold reduction of the initial concentration at a given temperature was determined by *Equation 4* (Mercali et al., 2013):

$$D = \frac{\ln 10}{K_d} \tag{4}$$

where K_d is the degradation rate constant (h^{-1}).

2.7 ARRHENIUS EQUATION

The Arrhenius equation relates the temperature to the constant for the speed of elementary reactions, and allows the determination of the activation energy and the frequency factor for pigment degradation reactions, as expressed by *Equation 5* (Fogler, 2012):

$$K_d = A \exp^{(-E_d/RT)} \tag{5}$$

where K_d is the degradation rate constant (s⁻¹), A is the frequency factor (s⁻¹), E_d is the activation energy for the degradation reaction (J mol⁻¹), T is the temperature (K) and R is the gas constant equal to 8.314 J mol⁻¹K⁻¹.

2.8 THERMODYNAMIC ANALYSIS

The activation enthalpy ΔH (J mol⁻¹), the free energy of inactivation ΔG (kJ mol⁻¹) and the activation entropy ΔS (J mol⁻¹K⁻¹), were calculated for each temperature studied and were obtained using *Equations 6, 7* and *8* (Labuza, 1980):

$$\Delta H = E_a - RT \tag{6}$$

$$\Delta G = -RT \ln\left(\frac{K_d h}{k_B T}\right) \tag{7}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{8}$$

where E_a is the activation energy for the degradation reaction (J/gmol), K_d is a degradation rate constant (s⁻¹), h is Planck's constant equal to 6.6262x10⁻³⁴ J s⁻¹, k_B is Boltzmann's constant equal to 1.3806x10⁻²³ J K⁻¹, T is the temperature (K), and R is the gas constant (J mol⁻¹K⁻¹).

2.9 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 degradation of betanin from *Beta vulgaris* L. between 50 and 80°C, followed first-order reaction kinetics (Figure 1) in agreement with the studies of and Saguy et al. (1979), Mabit et al. (2008), Gonçalves et al. (2013) and Shaaruddin et al. (2017). Since pH 5 provided the greatest stability to the betanin extract of *Beta vulgaris* L., this condition was mantained to analyze the effect of the addition of 40% sucrose and 10% fructose on thermodegradation between 50 and 80°C.

According 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.The high concentration of sugars in fruits preserves the stability of the pigments and this effect can be explained by the fact that high sugar concentration reduces water activity. Therefore, the incorporation of sucrose and fructose into the betanin extract could provide a gain in thermostability. Table 1 shows a comparison of the kinetics of degradation of the control betanin extract with that of extracts with added sugars.

The effects of the same sugar may be different due to the fact that there are differents matrices composition as well as on other food products properties and therefore, controversial results of the influence of sugars on stability have been reported (Rubbermann et al., 2006). Rubinskiene et al. (2005) reported that the addition of fructose at concentrations between 10 and 40% reduced the thermostability of the black currant anthocyanins extract while concentrations between 20 and 40% of sucrose were effective in increasing the half-life of the same extract.

For betanin extract, at 50°C, there was a gain in the half-life, however, given low coefficients of determination (below 0.9), there was no positive effect on the addition of the conservants. At 60°C, the additions of 40% of sucrose and 10% of fructose increased the half-life of the extract compared to the control extract in 26 and 20.7%, in that order. However, for the two higher temperatures, in accordance with the kinetic analysis, only the addition of sucrose was effective, especially at 70°C (22.6% increase in $t_{1/2}$), since there was no significant difference between the half-life values in relation

to that obtained for the control extract. This effect could be explained by the fact that sugar addition reduces water activity and it is known that low water activity stabilizes pigments like betanin. In the high water activity, the reactants are in solution and further dilution by water reduces the reaction rate by the law of mass action. So, according Karel & Lund (2003), the reaction rate is proportional to the reactant concentration which decreases with increasing water content. The opposite behavior was observed when fructose was added to the extract at 70 and 80°C. The combination of high temperatures and low inicial water activities may have caused the increase in the degradation rate constant

The inverse of the temperature was also correlated with the logarithm of the degradation rate constant using the Arrhenius equation and the activation energy was determined as 81.54±4.4, 87.96±2.35 and 102.345±2.41 J mol^{-1}, for extracts pure, with 40% of sucrose and with 10% of fructose, respectively.

Thermodynamic parameters such as enthalpy, free energy and entropy can also provide important information about thermal degradation kinetics.

According to Georgieva et al. (2012) activation enthalpy (ΔH) shows the energy difference between the reagent and activated complex. Similar values for all conditions evaluated indicates that the energy barrier that must be overcome in order to achieve the transition state is similar. The positive sign of ΔH means that anthocyanin degradation is an endothermic reaction, accompanied by the adsorption of heat.

Considering the free energy of inactivation (ΔG), which represents the difference between the activated state and the state of the reactants, the positive values indicated that betanin degradation is a non-spontaneous reaction and the similar values indicated similar degradation mechanisms under the different conditions for the same temperature. From the ΔH and ΔG values it was possible to determine the activation entropy (ΔS), that represents the change in disorder of molecules in the system and the negative values obtained show that that the molecules in the transition state are more organized than those at the initiation of the reaction, and therefore, formation of the activated complex is associated with a decrease in entropy (Labuza, 1980).

Thermodynamically (Table 2), the incorporation of fructose was more promising in increasing the stability of the betanin extract, since the ΔH values (for all temperatures) were higher than those obtained with sucrose. In this way, there is a need for a high energy barrier to break the bonds. Furthermore, the values obtained for ΔS in this study were low, which, according to Georgieva et al. (2012), means that the state of the material is close to its own thermodynamic equilibrium and thus the material shows little reactivity and has undergone some kind of physical or chemical rearrangement in its initial structure, bringing it to a state close to its own thermodynamic equilibrium.
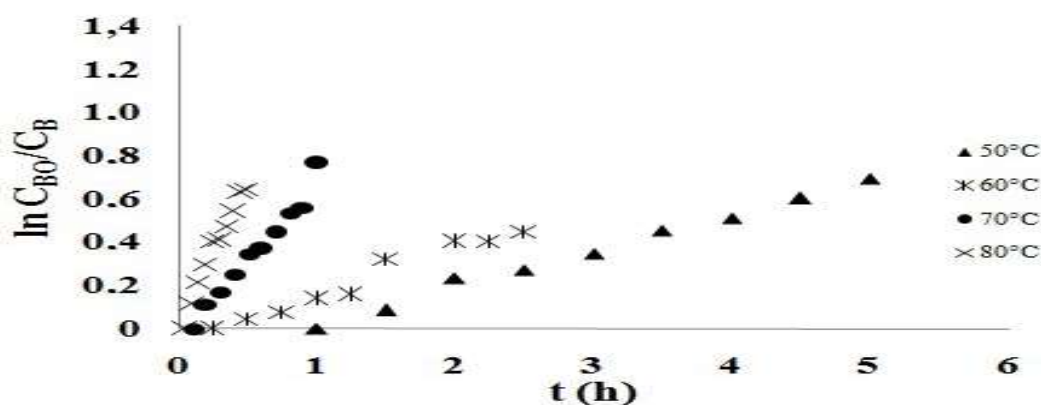

FIGURE 1 - DEGRADATION OF BETANIN FROM *BETA VULGARIS* **L. DURING HEATING BETWEEN 50 AND 80°C AT PH 5. EACH POINT REPRESENTS THE AVERAGE OF 3 REPLICATES AND THE STANDARD ERROR OF EACH POINT IS AROUND 5%.**

*C_{BO} is initial betanin concentration (mg 100 g red beet[-1]), C_B is concentration immediately after sample collection (mg 100 g red beet[-1]) and t is time (h).

TABLE 1 - EFFECT OF SUCROSE AND FRUCTOSE IN K_D, $T_{1/2}$ AND D-VALUES ON BETANIN DEGRADATION

Sample	T (K)	K_d (h^{-1})**	$t_{1/2}$ (h)	D (h)
Control	323	0.09±0.01 (0.83)	7.7	25.6
	333	0.51± 0.007 (0.99)	1.35	4.47
	343	0.75± 0,059(0.98)	0.93	3.08
	353	1.37±0.04 (0.94)	0.51	1.68
40% of sucrose	323	0.07±0.0036 (0.75)	10.13	33.66
	333	0.41±0.009 (0.93)	1.7	5.66
	343	0.61±0.02 (0.93)	1.14	3.78
	353	1.3±0.03 (0.94)	0.53	1.78
10% of fructose	323	0.06±0.004 (0.84)	12	39.98
	333	0.42±0.152 (0.98)	1.63	5.42
	343	0.82±0.031 (0.95)	0.84	2.79
	353	1.65±0.06 (0.95)	0.42	1.4

*Kd: degradation rate constant;
$t_{1/2}$: half-life;
D: decimal reduction time;
**Numbers in parentheses are the correlation coefficients.

TABLE 2 - THERMODYNAMIC PARAMETERS OBTAINED FOR BETANIN DEGRADATION WITH SUGARS ADDICTION

Sample	T (K)	ΔH (J mol^{-1})	ΔG (kJ mol^{-1})	-ΔS (J mol^{-1}K^{-1})
Control	323	78.85	107.78	0.09
	333	78.77	106.37	0.08
	343	78.69	108.58	0.09
	353	78.61	110.06	0.09
40% of sucrose	323	85.27	108.51	0.07
	333	85.19	107.02	0.06
	343	85.11	109.17	0.07
	353	85.03	110.22	0.07
10% of fructose	323	99.66	108.98	0.03
	333	99.58	106.90	0.02
	343	99.49	108.31	0.02
	353	99.41	109.51	0.03

*T: temperature;
ΔH: activation enthalpy;
ΔG: free energy of inactivation;
ΔS: activation entropy.

4 CONCLUSIONS

Addition of 10% of fructose and 40% of sucrose had a positive effect on the stability of betanin, especially at lower temperatures between 50 and 80°C, leading to an increase of the half-life value of the extract by up to 26% and consequently increase of the D value and decrease of K_d. This effect could be explained by the fact that sugar addition reduces water activity and low water activity stabilizes pigments like betanin. The degradation of the extract under all conditions followed a first-order kinetic model and according the ΔG and ΔH degradation reaction is non-spontaneous and exothermic. From a thermodynamic point of view, fructose promoted stability more successfully than sucrose because the ΔH values were higher than those obtained with sucrose and the low values of ΔS when incorporated this sugar means that the state of the material is close to its own thermodynamic equilibrium.

ABSTRACT

EFFECTS OF THE ADDITION OF SUGARS ON THE THERMOSTABILITY OF BETANIN FROM *BETA VULGARIS* L.

The impact of the addition of sugars on stability of betanin from *Beta vulgaris* L. (red beet) by thermal treatment between 50 and 80°C and pH 5 were investigated. The degradation of betanin pure and added by 40% of sucrose and 10% of fructose followed a first-order kinetic model and as the temperature was increased, there was an increase in degradation rate constant values (Kd), a reduction in the half-life and the values of decimal reduction time (D). Thermodynamically, the incorporation of fructose was more promising in increasing the stability of the betanin extract, since the activation enthalpy values (ΔH) were higher (between 99.66 and 99.41 kJ mol^{-1}) than those obtained with sucrose (between 85.27 and 85.03 kJ mol^{-1}) and pure (between 78.85 and 78.61 kJ mol^{-1}). The positive values of free energy of inactivation (ΔG) indicated that betanin degradation is a non-spontaneous reaction and the low values of activation entropy (ΔS) to extract with fructose (between 0.02 and 0.03 J mol^{-1}K^{-1}) means that the state of the material is close to its own thermodynamic equilibrium.

KEYWORDS: *STABILITY, THERMODYNAMIC FUNCTIONS, SUCROSE, FRUCTOSE, NATURAL PIGMENT*

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