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EVALUATION AND OPTIMIZATION OF NON ENZYMATIC BROWNING OF "CAJUINA" DURING THERMAL TREATMENT

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Abstract - "Cajuina" is a very popular drink in the Brazilian northeastern region and is produced by clarifying cashew apple juice. To preserve "cajuina" from chancing, the clarified cashew apple juice is submitted to thermal treatment where a desired final color should be obtained. To optimize color formation while maintaining high vitamin C and low 5-hydroxymethylfurfural (5-HMF) concentrations the thermal treatment of "cajuina" needs to be studied and the non enzymatic mechanism should be better understood and controlled. In this work the effect of thermal treatment on "cajuina" (clarified cashew apple juice) was studied at temperatures from 88°C to 121°C. Changes in color were measured and the variation in vitamin C, 5-hydroxymethylfurfural (5-HMF) and sugar content were used to evaluate non enzymatic browning. The kinetic models were used to optimize the thermal treatment to produce "cajuina" with an absorbance at 420 nm of 0.023.
Keywords: Cajuina; Non enzymatic browning; Thermal treatment; Optimization.

INTRODUCTION

Food products from the cashew tree (*Anacardium occidentale*) can be divided into two groups, one the cashew nut, the real fruit, and other the fruit peduncle from which juice, candies and other products can be produced. Cashew apple juice has a pleasant flavor and is rich in vitamin C, but has limited acceptance due to its astringency. Clarified cashew apple juice, however, has greater acceptance due to its low astringency.

Thermal treatment is used to preserve fruit derivatives in the manufacturing process. Negative effects of thermal treatments include non enzymatic browning, loss of nutrient and formation of undesirable products such as 5-hydroxymethylfurfural (5-HMF). Browning due to thermal treatment is the result of several reactions known as Maillard reactions, which include condensation between reducing sugars and amino acids, caramellization, ascorbic acid browning and pigment destruction (Cornwell & Wrolstad, 1981; Beveridge, Franz, & Harrison, 1986). Non enzymatic browning reactions mainly cause color change, sugar and vitamin C loss and 5-HMF formation, affecting the quality of fruit juices (Ibarz, Pagán, & Garza, 1999). To control browning and preserve the quality of the juice, the reactions that cause non enzymatic browning need to be studied and the main factor involved needs to be identified. For the design process and to optimize

*To whom correspondence should be addressed process conditions adequate kinetic models of the reactions are required.

The objective of this study was to discover the main reaction that causes non enzymatic browning in “cajuina” submitted to thermal treatment at high temperatures. Studies on colorimetric parameters and on the evolution of sugar and ascorbic acid (vitamin C) content as well as on 5-HMF formation were carried out to obtain suitable kinetic models for the reactions. The kinetic models were used to optimize thermal treatment of “cajuina” to render a product with an absorbance at 420 nm of 0.023, a high vitamin C content and a low 5-HMF concentration.

MATERIALS AND METHODS

Cashew Apple Juice

Cashew apples were collected at Embrapa Experimental Station (Pacajus - CE, Brazil). The cashew apples were washed in running water and pressed to obtain the juice in an expeller press (Incomap 300). The cashew apple juice was clarified by with addition of food grade gelatin solution followed by filtration. After clarification the juice was bottled.

Thermal Treatment

Thermal treatment was carried out on clarified cashew juice samples at four different temperatures, 88, 100, 111 and 121ºC. The experiments at 88 and 100ºC were carried out in thermal water bath equipment (Fanem model 147) and the experiments at 111 and 121ºC were carried out in an autoclave (Quimis model Q-190-24). Aliquots were extracted at different time intervals for each temperature and immediately brought to room temperature in an ice-water bath. Chemical and colorimetric determinations were performed for each aliquot. Experiments and analysis were carried out in triplicate.

Physical and Chemical Analysis

Variation in absorbance at 420 nm (A_{420}) was measured using a Cary50conc UV-VIS spectrophotometer. Vitamin C was measured by the diclorofenol-2,6-indofenol method in accordance with Strohecker and Henning (1967) using a Cary50conc UV-VIS spectrophotometer at 520nm. Soluble solids content was determined with an Atago PR-101 refractometer.

Glucose, fructose, sucrose and 5-HMF were determined by HPLC using a Varian ProStar. For sugar analysis, water was used as mobile phase and a refractive index detector with a Varian Metacarb 87P (300 mm × 7.8 mm) column was used. For 5-HMF, a mixture of acetonitrile:water (20:80) and a UV-visible detector fixed at a wavelength of 285 nm with a Varian Microsorb (C-18) column were used.

Mathematical Modeling

In non enzymatic browning reactions there is an initial induction period which corresponds to the stage of colored-compound formation. After this induction period, which can be fast, the color of the product increases linearly with time (zero-order kinetics) or exponentially (first-order kinetics) (Labuza, 1972; Toribio & Lozeno, 1984; Garza, Ibarz, Pagán, & Giner, 1999).

$$C = C_0 + k_0 \cdot t \quad \text{(Zero-order kinetics)} \qquad (1)$$

$$C = C_0 \cdot \exp\left(k_1 \cdot t\right) \quad \text{(First-order kinetics)} \qquad (2)$$

Production of colored compounds requires the reaction and hence consumption of key juice components such as sugars, amino acids and ascorbic acid. The consumption of these compounds can also follow a zero-order or first-order kinetics and were also modeled with equations 2 and 3. Regression analysis (curve fitting) and calculation of kinetic rate constants were performed using Microcal Origin v.6.0 software. Statistical analysis of the regression and goodness of fit was done using Statistica v5.0 software. All statistical analysis were carried out at a 95% confidence level.

RESULTS AND DISCUSSION

Table 1 contains the results of the physicochemical characterization obtained for the clarified cashew apple juice prior to thermal treatment. The physicochemical characterization of the cashew apple was similar to that reported in the literature (Azoubel et al., 2005).

Non enzymatic browning has several causes such as reaction of reducing sugars with amino acids, sugar caramellization, vitamin C decomposition and pigment destruction. The main causes of browning differ with different juices, so it is important to discover which factor most affects browning in cashew apple juice.

Table 1: Physicochemical characteristics of clarified cashew apple juice.

Absorbance at 420 nm	0.0111
Soluble solids content [ºBrix]	12.2
Reducing sugars [g/100g]	9.8
Total sugars [g/100g]	9.9
pH	4.4
Ascorbic acid [mg/L]	179.8
5-HMF [mg/L]	0.0

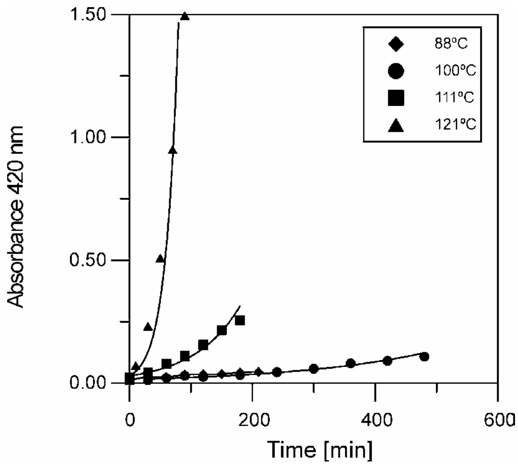

Figure 1: Evolution of the absorbance at 420 nm of clarified cashew apple juice during thermal treatment at different temperatures.

The changes in absorbance at 420 nm (A_{420}) were studied with time of treatment. Results showed that increasing processing time increased the absorbance at 420 nm (Fig. 1). Increasing temperature was also shown to increase browning rate measured by A_{420}. The A_{420} variation was adequately described by a first-order kinetic model and the kinetic rate constant followed the Arrhenius equation:

$$\frac{dA_{420}}{dt} = +k_{420} \cdot A_{420} \quad (3)$$

$$A_{420} = A_{420}^{0} \cdot \exp\left(k_{420} \cdot t\right) \quad (4)$$

$$k_{420} = 6.978 \times 10^{29} \cdot \exp\left(\frac{-28752}{T}\right) \quad (5)$$

At zero time measurement of the change in absorbance at 420 nm already registered an initial reading (A_{420}^{0} = 0.0111), which was due to compounds inherent in the juice.

The concentration of reducing sugars, fructose and total sugars as well as soluble solids content did not change with time and the variation among data points was within the standard error (Fig. 2). The results obtained for total sugars and reducing sugars did not show any definite tendency at any temperature studied, and the steady concentration of sugars during thermal treatment showed that sugars did not react with amino acids and therefore did not affect browning.

Increasing processing time and temperature had a significant effect on decomposition of ascorbic acid (Fig. 3). The change in ascorbic acid was adequately described by a first-order kinetic model and the kinetic rate constant followed the Arrhenius equation:

$$\frac{dAA}{dt} = -k_{AA} \cdot AA \quad (6)$$

$$AA = AA^{0} \cdot \exp\left(-k_{AA} \cdot t\right) \quad (7)$$

$$k_{AA} = 2.134 \times 10^{12} \cdot \exp\left(\frac{-13081}{T}\right) \quad (8)$$

Correlation of the change in absorbance at 420 nm with loss of ascorbic acid showed an inverse relationship, indicating that ascorbic acid may be the main factor that causes browning in clarified cashew apple juice. This is in accordance with several proposed theories that implicate in loss of ascorbic acid, the formation of browning products such as furan-type compounds, lactones, acids, 3-hydroxy-2-pyrone, furaldehyde and 5-hydroxymethylfuraldehyde (Clegg, 1964; Clegg & Morton, 1965; Tatum, Shaw, & Berry, 1969; Kanner, Harel, Fishbein, & Shalom, 1981; Robertson & Samaniego, 1986). A number of these compounds, identified as non enzymatic browning products, had already been found in fruit juices (Roig, Bello, Rivera, & Kennedy, 1999).

The concentration of 5-HMF was affected by processing time and temperature, showing two distinct kinetic rates: a first-order kinetic rate at the beginning of the thermal treatment and a zero-order kinetic rate at later processing times (Fig. 4). The transition between the first-order kinetics and the zero-order kinetics was also temperature dependent. The first kinetic period of 5-HMF was related to decomposition of ascorbic acid producing 5-HMF. The effect of ascorbic acid decomposition on the formation of 5-HMF decreased as the concentration of ascorbic acid decreased to less than 120 mg/L and the second kinetic period started. The second kinetic period might be affected by a combined effect of ascorbic acid degradation and sugar caramellization, which can slowly produce 5-HMF. The experiments carried out at 88ºC did not show measurable amounts of 5-HMF due to the slow rate of this reaction at low temperatures.

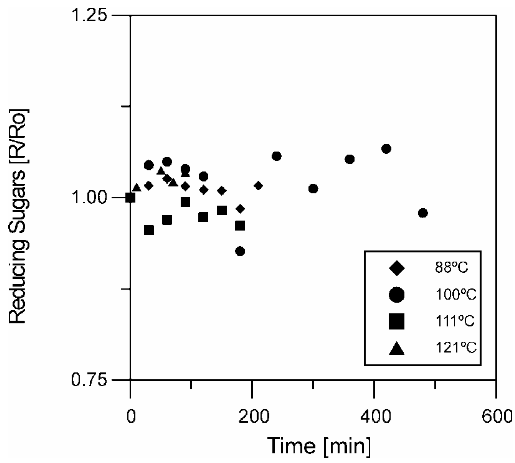

Figure 2: Evolution of the reducing sugars of clarified cashew apple juice during thermal treatment at different temperatures.

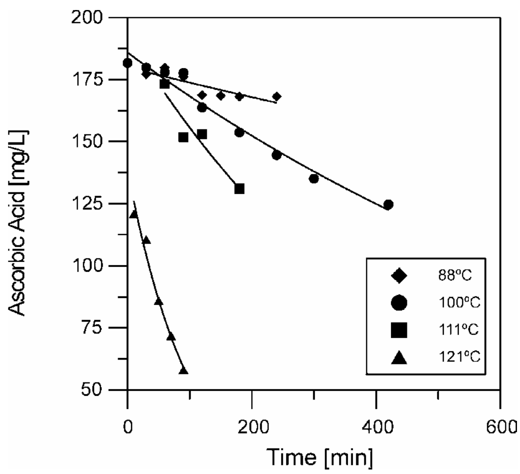

Figure 3: Evolution of the ascorbic acid of clarified cashew apple juice during thermal treatment at different temperatures.

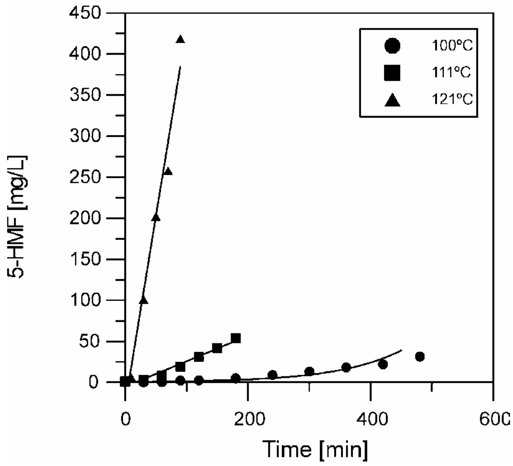

Figure 4: Evolution of the 5-hydroxymethylfurfural (5-HMF) of clarified cashew apple juice during thermal treatment at different temperatures.

The first kinetic period was adequately described by a first-order kinetic model and the kinetic rate constant followed the Arrhenius equation:

$$\frac{dHMF}{dt} = +k_{HMF} \cdot HMF \quad (9)$$

$$HMF = HMF^0 \cdot \exp\left(k_{HMF} \cdot t\right) \quad (10)$$

$$k_{HMF} = 8.874 \times 10^{25} \cdot \exp\left(\frac{-24274}{T}\right) \quad (11)$$

$$HMF^0 = 1.139 + 2.170 \times 10^{-39} \cdot \exp\left(\frac{33105}{T}\right) \quad (12)$$

The second kinetic period was described by a zero-order kinetic model. The kinetic rate constant showed a linear relationship with temperature:

$$\frac{dHMF}{dt} = +k_{HMF,2} \quad (13)$$

$$HMF = HMF_2^0 + k_{HMF,2} \cdot t \quad (14)$$

$$k_{HMF,2} = -178.40 + 0.4655 \cdot T \quad (15)$$

$$HMF_2^0 = 1647.82 - 4.328 \cdot T \quad (16)$$

The transition period was observed in the thermal treatments at 111 and 121ºC and the transition time could be adequately fit by the equation:

$$t_{TR} = 30 + 5.320 \times 10^{34} \cdot \exp\left(\frac{T}{5.051}\right) \quad (17)$$

The regression coefficients obtained were 0.958 for absorption, 0.978 for ascorbic acid and 0.949 for 5-HMF concentration. These values indicate that the curve fittings were satisfactory, especially when dealing with fruits which may have some variation in initial chemical composition.

Process Optimization

Process optimization requires understanding the preferences of consumers, who opt for a dark brown "cajuina" characterized by absorbance at 420 nm of 0.023, maintaining a high nutritional value characterized by high vitamin C content (Embrapa, 2005). To meet customers expectations the thermal treatment needs to increase the clarified cashew apple juice absorbance at 420 nm by 109%. Thus, absorbance at 420 nm acts as a constraint to process optimization, which will have two degrees of freedom: time and temperature. Having two degrees of freedom means that the process can have multiple optimum operating conditions represented by time-temperature sets as shown in Fig. 5.

According to the International Federation of Fruit Juice Producers (IFFJP, 1985) the recommended maximum 5-HMF concentration in fruit juices is 5 mg/L, so the 5-HMF concentration has to be evaluated at all optimum time-temperature sets that were obtained. If a time-temperature set produces more 5-HMF than the concentration limit then this time-temperature set cannot be employed to produce "cajuina". Fig. 6 shows 5-HMF concentration as a function of temperature calculated at the optimum processing time for that temperature. The concentration of 5-HMF did not exceed the maximum recommended concentration for any thermal treatment temperature and decreased as treatment temperature increased. Higher temperatures resulted in higher 5-HMF formation rates, but the significantly lower processing time required at higher processing temperatures had a greater effect on the process, decreasing the 5-HMF concentration.

Vitamin C content can act as a constraint on finding a single optimum operating condition. There is no recommended value for vitamin C concentration in juices, but it is desirable to lose as little vitamin C as possible. In Fig. 7 ascorbic acid content as a function of temperature, calculated at the optimum processing time for that temperature, is shown. The concentration of ascorbic acid is very dependent on processing time and process temperature. As temperature increases vitamin C content diminishes up to a temperature of 105ºC, when the optimal processing time becomes so short that the degradation of ascorbic acid is low. Therefore, based on vitamin C concentration, the best thermal treatment would be carried out at 120ºC, the temperature which results in the highest vitamin C concentration in the clarified cashew apple juice.

If the thermal treatment is carried out using a water bath under atmospheric pressure, then the maximum temperature that can be achieve is 100ºC. In this case the best thermal treatment would be carried out at 90ºC, a temperature that would maintain a high concentration of vitamin C in the juice.

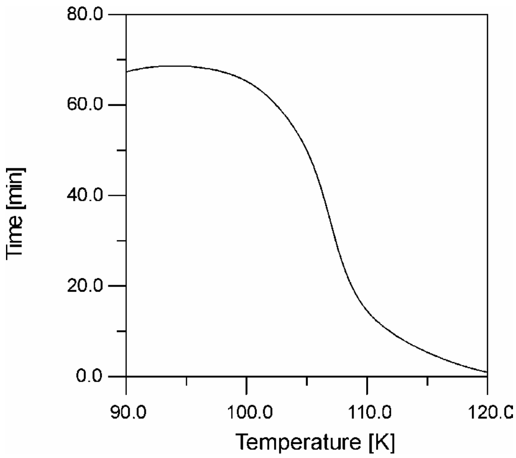

Figure 5: Possible optimal time-temperature processing conditions to achieve an absorbance at 420 nm 10% higher than the initial absorbance of the juice at 420 nm.

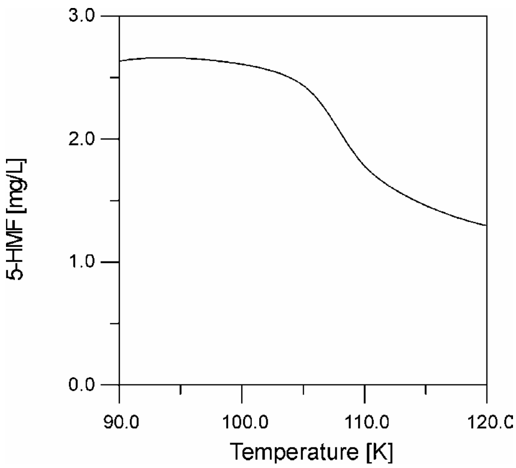

Figure 6: 5-HMF concentration found at the optimal operating time for different processing temperatures.

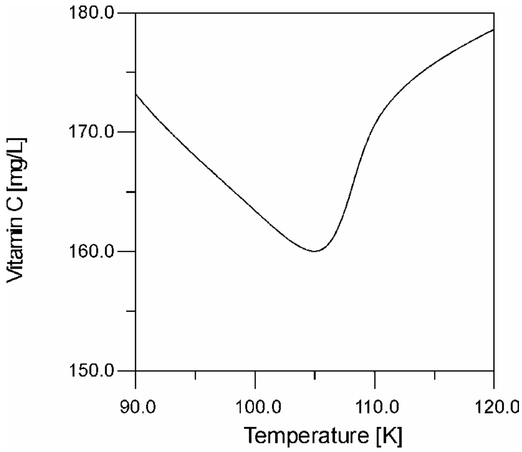

Figure 7: Ascorbic acid (vitamin C) concentration found at the optimal operating time for different processing temperatures.

CONCLUSIONS

Changes in absorbance at 420 nm and ascorbic acid during thermal treatment of clarified cashew apple juice could be described by first-order kinetics, showing the correlation between loss of ascorbic acid and color formation (browning). Formation of 5-HMF had two kinetic mechanisms and the first stage followed first-order kinetics in direct association with loss of ascorbic acid. Sugar degradation was not observed and thus may not be related to the browning of cashew apple juice.

Optimization of the process based on high Vitamin C content showed that the thermal treatment should be carried out at 120ºC and low residence times in plate heat exchangers or similar heat-transfer equipment. If a water bath is used as the thermal treatment equipment, then the process should be carried out at 90ºC, a temperature at which the degradation of ascorbic acid is lower, but at the expense of a higher 5-HMF content.

The kinetics of vitamin C degradation and its correlation with color formation are important to optimize the color aspect of the industrial production of "cajuina", which is still not carried out under optimized conditions. Although the optimization carried out in this work was done aiming at an absorbance at 420 nm of 0.023, the kinetics obtained and optimization procedure can be used to optimize the production of "cajuina" with either a lighter or a darker color.

NOMENCLATURE

A_{420}	absorbance at 420 nm	(-)
A_{420}^{0}	initial absorbance at 420 nm	at t = 0
AA	concentration of ascorbic acid	mg/L
AA_0	initial concentration of ascorbic acid (at t = 0)	mg/L
C	process variable	(-)
C_0	initial value of the process variable	(-)
HMF	concentration of 5-HMF	mg/L
HMF_0	initial concentration of 5-HMF (at t = 0)	mg/L
HMF_2^{0}	initial concentration of 5-HMF at the beginning of the second kinetic stage	mg/L
k_0	process variable zero-order kinetic rate constant	(-)
k_1	process variable first-order kinetic rate constant	(-)
k_{420}	absorbance kinetic rate constant	min^{-1}
k_{AA}	ascorbic acid degradation kinetic rate constant	min^{-1}
k_{HMF}	5-HMF formation first-order kinetic rate constant (first kinetic stage)	min^{-1}
$k_{HMF,2}$	5-HMF formation zero-order kinetic rate constant (second kinetic stage)	min^{-1}

t	time	min
t_{TR}	transition time between the first and second kinetic mechanisms of 5-HMF formation	Min
T	temperature	K

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