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Chemical and physicochemical stability of beverage based integral cashew apple and honey

Estabilidade química e físico-química de bebida a base de caju integral e mel

Estabilidad química y físico-química de bebidas a base de anacardo integral y miel

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Abstract

This work aimed to study beverages' storage stability based on cashew apple pulp and honey during 120 days. Three formulations were prepared: F1 (75% of cashew apple pulp and 25% of honey; F2 (60% of cashew apple pulp and 40% of honey) and F3 (50% of cashew apple pulp and 50% of honey). The formulation F1 showed the highest content of ascorbic acid after storage (95.74 mg.100mL). The pH did not show variation between the formulations studied. Soluble solids showed significant differences during storage and were consistent with the drink's honey content, ranging from 28.8 to 30.0 for F1, from 36.5 to 40.1 for F2, and 44.3 to 47.7 for F3. The content of carotenoids and phenolic compounds decreased during storage time. The preparation of beverages based on cashew apple and honey presented an alternative to adding value to the cashew apple pulp.

Keywords: *Anacardium occidentale* L.; Peduncle; Honey; Shelf life; Physicochemical properties; Beverages; Integral juice.

Resumo

Este trabalho teve como objetivo estudar a estabilidade de armazenamento de bebidas à base de polpa de caju e mel durante 120 dias. Foram preparadas três formulações: F1 (75% da polpa do caju e 25% do mel; F2 (60% da polpa do caju e 40% do mel) e F3 (50% da polpa do caju e 50% do mel). A formulação F1 apresentou o maior teor de ácido ascórbico após o armazenamento (95,74 mg.100mL). O pH não apresentou variação entre as formulações estudadas. Os sólidos solúveis apresentaram diferenças significativas durante o armazenamento e foram compatíveis com o teor de mel da bebida, variando de 28,8 a 30,0 para F1, de 36,5 para 40,1 para F2 e de 44,3 para 47,7 para F3. O teor de carotenóides e compostos fenólicos diminuiu durante o armazenamento. O preparo de bebidas à base de caju e mel apresentou uma alternativa para agregar valor à polpa de caju.

Palavras-chave: *Anacardium occidentale L.*; Pedúnculo; Mel; Vida útil; Propriedades físico-químicas; Bebidas; Suco integral.

Resumen

Este trabajo tuvo como objetivo estudiar la estabilidad de almacenamiento de bebidas a base de pulpa de anacardo y miel durante 120 días. Se prepararon tres formulaciones: F1 (75% de pulpa de anacardo y 25% de miel; F2 (60% de pulpa de anacardo y 40% de miel) y F3 (50% de pulpa de anacardo y 50% de miel). La formulación F1 mostró el mayor contenido de ácido ascórbico después del almacenamiento (95.74 mg. 100mL). El pH no mostró variación entre las

formulaciones estudiadas. Los sólidos solubles mostraron diferencias significativas durante el almacenamiento y fueron consistentes con el contenido de miel de la bebida, que van de 28.8 a 30.0 para F1, de 36.5 a 40.1 para F2 y de 44.3 a 47.7 para F3 El contenido de carotenoides y compuestos fenólicos disminuyó durante el tiempo de almacenamiento La preparación de bebidas a base de anacardo y miel presentó una alternativa para agregar valor a la pulpa de anacardo.

Palabras clave: *Anacardium occidentale* L.; Pedúnculo; Miel; Vida útil; Propiedades fisicoquímicas; Bebidas; Jugo integral.

1. Introduction

There is an increasing demand from the consumers for a greater variety of fruits in their diets. This concern is not only for fresh tropical fruits but also for their juices. The impact of this demand in the developed countries has promoted production and processing capacity, assuring the offering of these products in the international market (Cairns et al., 2020, Silva e Claro, 2019, Sugerman et al., 2011, Maia et al., 2009).

The cashew apple (*Anacardium occidentale* L) is one of the fruits with more expressive production in Brazil. However, less than 20% of the production is used in the fruit juice industry. The cashew apple is a rich source of vitamin C, carotenoids, and phenolic compounds. Its primary utilization is in juice, but its high astringency affects its acceptance (Sousa et al., 2007; Sousa et al., 2010). Its consumption is usually diluted and sweetened.

Besides the fruit juices' nutritional and functional properties, it is interesting to add functional properties components to produce functional drinks. The utilization of various elements with functional and/or medicinal action should be studied, and the product being consumed in conventional diets. Due to its therapeutic importance and a natural sweetener, honey is an alternative to sweeten cashew pulp drinks and serve a market niche formed by sportsmen and consumers, presenting them with healthier food by replacing sucrose with honey (Anjos et al., 2020).

The honey has low pH (3.3-4.0) (Nanda et al., 2009; Feás et al., 2010), energetic action, enzymes, vitamins, and minerals. The honey processes the majority of the essential minerals for the human organism, especially selenium, manganese, zinc, chromium, and aluminum (Silva et al., 2006).

The purpose of the present work was to study the storage stability during 120 days of formulations based on integral cashew apple (*Anacardium occidentale* L.) pulp added with

honey (*Apis melifera*) in different proportions.

2. Materials and Methods

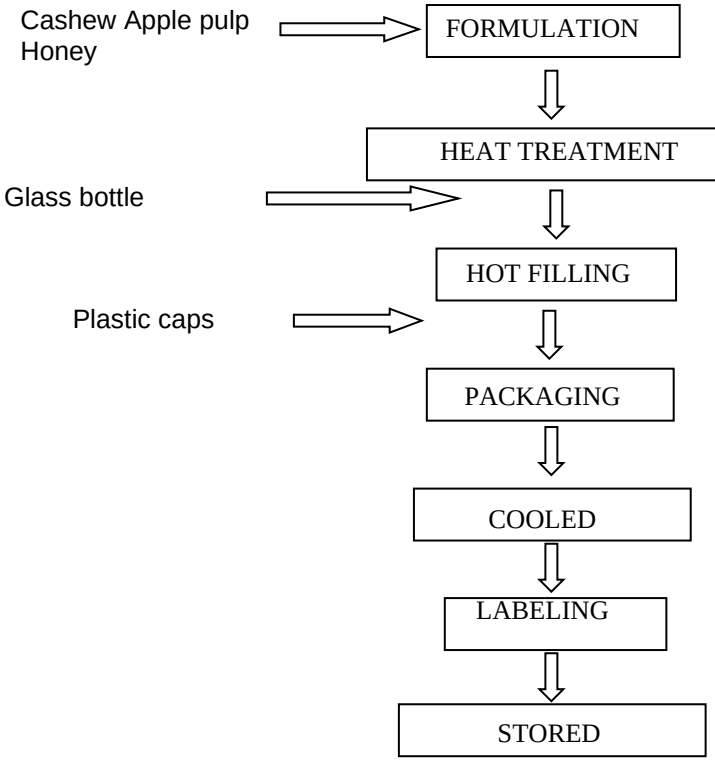
The present study used quantitative analysis to evaluate the data using mathematical methods and qualitative to interpret the results (Pereira et al., 2018).

In this work, it was used pasteurized cashew apple pulp (*Anacardium occidentale* L.) donated by a local fruit processing industry, and honey (*Apis melifera*) of floral origin, black Jurema (*Mimosa tenuiflora*) acquired in the local market. The investigation regarding the bloom's botanical origin was achieved at the Apiculture Laboratory - Federal University of Ceara - Brazil through the honey samples' melissopalinologic analysis in an optical microscope the methodology described by Barth (1989).

The amounts of cashew apple pulp and honey were established according to the final dilution of the product when it was done a mass balance to obtain a final product with a solids soluble value of 11,5 ºBrix, according to the Codex Alimentarius regulation (CODEX ALIMENTARIUS, 2005). The three obtained formulations were as follows F1 (75% of cashew apple pulp and 25% of honey), F2 (60% of cashew apple pulp and 40% of honey), and F3 (50% of cashew apple pulp and 50% of honey).

In the formulation step, the cashew apple and honey were adjusted and submitted to heat treatment at 90 ºC/60s, followed by hot filling in 250mL glass bottles previously sterilized and closed with plastic caps. After the packaging, the bottles containing the beverages were immediately cooled in running water. The beverages were stored on shelves at room temperature (25 º ± 2 ºC) for 120 days (Figure 1).

Figure 1 - Fluxogram for processing the formulated beverages base on cashew apple pulp and honey.



Source: Authors (2020).

Chemical and Physicochemical determinations

The chemical and physicochemical analysis of the raw material and formulated beverages were effected in duplicates, consisting of the following analysis: 1 - pH, determined by phmeter HANNA INSTRUMENTS, model HI 9321; 2 - soluble solids (^{o}Brix) it was used a refractometer ATAGO provided with scale varying from 0 to 90 ^{o}Brix; 3 - total acidity, determined by titration according to the methodology Instituto Adolfo Lutz (2008), using NaOH 0.1Mol.L^{-1} and phenolphthalein as indicator, and the results were expressed as g of citric acid.100 mL^{-1} of the sample or meq.Kg^{-1}; 3 - reducing and total sugars determined by spectrophotometry, using a spectrophotometer UV-vis (Micronal, model B582) at 540 nm, according to Miller (1959). It was used 3,5-dinitro-salicylic acid (DNS). In the determination of total sugars, previous acid inversion of the extracts were effected using cloridric acid, and the results were expressed as grams of glucose.100mL^{-1} of juice (Instituto Adolfo Lutz, 2008); 4 - The browning of the beverages was determined through the analysis of water-soluble pigments according to Rattanathanalerk et al. (2005). Fifteen mL of the samples were

centrifuged at 3000 rpm for ten minutes. To 5 mL of the supernatant, 5 mL of ethylic alcohol was added, followed by new centrifugation. For the readings, a spectrophotometer UV-vis (Micronal, Model B582) was used at 420nm. For color determination, a colorimeter Minolta (Model Chroma-Meter CR410) with values expressed as L*, a* and b*. Numeric values of a and b were converted in Hue angle and Chroma through the formulas Hue angle = tan b*/a* and Chroma = (a* + b*); 5 - The ascorbic acid content was determined by titration based on the reduction of the indicator 2,6-dichlorophenol indophenol by the ascorbic acid being expressed as mg of ascorbic acid.l00mL^{-1} of sample (IAL, 2008); 6 - The total carotenoids were extracted with 1 mL of sample and 10 mL of extractor solution acetone:hexane (4:6) in test tubes and homogenized for 1 minute. The readings were effected in spectrophotometer UV-vis (Micronal, Model B582) using the wave lengths 453nm, 505nm, 645nm, 663nm. The results were expressed as µg of beta carotene .100mL^{-1} through the equation: Concentration = 0.216. A - 1.22.A – 0.304./ A + 0.452.A (Nagata and Yamashita, 1992); 7 - The phenolic compounds were determined by the methodology described by Reynertson et al. (2008) with modifications, using the Folin-Ciocalteau reagent. The samples previously homogenized were taken 2 mL, diluted in 20 mL ethanol 50%, allowed to stand for one hour, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant was filtered to a 50 mL volumetric flask, and to the residue was added 20mL of acetone 70%, allowing to stand for one hour and centrifuged. The supernatant was added to the volumetric flask; the volume was completed with distilled water. To determine the total phenolics, it was used 100 µL of the extract to which was added 1mL of the Folin ciocalteau 0.2 Mol.L^{-1} followed by homogenization in an agitator provided with Vortex tubes. After standing for 5 min it was added 1 µL of a 10% sodium carbonate. After one hour at room temperature, the extracts' absorbance was read at 765 nm in a spectrophotometer UV-vis (Micronal, Model B582). The formulated beverages were assayed in duplicates and quantified through a standard curve of gallic acid (0.001 to 0.2 mg of gallic acid. mL^{-1}). The results were expressed in mg of gallic acid.mL^{-1}.

Experimental and statistical analysis of data

The experiment was conducted according to the planning, in subdivided parcels with the beverages formulations in parcels realized in randomized order, with three levels (Formulation F1, Formulation F2, and Formulation F3) and storage time in the sub parcels in five levels (0, 30, 60, 90 and 120 days) maintained at room temperature (25º ± 2º C) in factorial completely at random. The assays were conducted with three repetitions.

The chemical and physicochemical data were subjected to an interaction between formulations and storage time and regression when suitable. The Tukey test was used for average comparison at 5% probability with the statistical program XLSTAT 2020.

3. Results and discussion

Characterization of the raw material

Table 1 shows the results of the chemical and physicochemical parameters of pH, titratable acidity, soluble solids, ascorbic acid, reducing sugars, total and total phenolic sugars from cashew pulp and bee honey used in formulated drinks.

Table 1 - Physico-chemical characterization of raw materials, cashew pulp, and bee honey, used in the experiment.

Determinations	**Raw**	
	Honey	**Cashew pulp**
Ascorbic acid	5.75	169.54
Titratable acid (% citric acid)	-	0.46
Free acid (meq.Kg^{-1})	29.1	-
pH	3.69	3.97
Soluble solids (^{o}Brix)	-	12.0
Total sugars (%)	83.32	9.38
Reducing sugars (%)	79.78	9.135
Total phenolics (mg AGE/mL)	1.99	2.72

Source: Authors (2020).

The results are within the ranges established by Brazilian legislation for cashew pulps (Brasil, 2000a).

The value found for ascorbic acid was 169.54 mg / 100mL of cashew pulp. According to different authors, there is a wide variation in vitamin C content: 253.28 mg / 100g of pulp (FIGUEIREDO et al., 2007), 112.1mg / 100g of pulp (Brandão et al., 2003). This variation can be attributed to several factors, such as soil type, harvest time, a form of cultivation, climate, type of cashew, and storage procedure (Assunção; Mercadante, 2003).

According to the current Brazilian legislation (Brasil, 2005) and Fao/Who (2001), the

recommended daily intake index (IDR) for vitamin C is 45mg. Thus, it is clear that 100g of cashew pulp already exceeds the necessary vitamin C index per day, proving that fruits are a great source of this vitamin.

The average pH values found in this experiment are below 4.5, which limits the development of microorganisms. Similar values were found by Assunção and Mercadante (2003), in which he saw a pH ranging between 3.8 and 4.5.

The average value of titratable acidity found in the cashew pulp was 0.46% citric acid. Values reported by Brandão et al. (2003) were very similar to those found in this experiment.

The pH value for the bee honey found was 3.69. The value found is similar to that obtained by Alves (2008) in samples of organic honey from African bees from the islands Floresta and Laranjeira, from the Upper Paraná River, which found a pH ranging from 3.33 to 4.04 in 24 analyzed samples. Anacleto-Almeida et al. (2004), in samples of honeys from the cerrado of São Paulo, found an average pH of 3.89.

Although pH is not currently indicated as a mandatory analysis in Brazilian honeys' quality control, it is useful as an auxiliary variable for quality assessment (SILVA et al., 2004). According to Crane (1985), honey's pH value can be influenced by the pH of the nectar, soil, or association of vegetables for honey composition.

The average acidity value found for bee honey was 29.1 meq.Kg^{-1}. Aroucha (2008) found values for acidity ranging from 31.25 to 86.75 meq.Kg^{-1}. Almeida (2002) detected in samples of honeys produced in São Paulo acidity ranging from 6.0 and 46.0 meq.Kg^{-1}. The legislation accepts maximum acidity of 50 meq.Kg^{-1} of honey (BRASIL, 2000b).

According to Nogueira-Neto (1997), the source of acidity in honey is due to the variation of organic acids, caused by different sources of nectar, by the action of the enzyme glucose-oxidase on the glucose that originates the glyconic acid.

The value of reducing sugars for the honey used was 79.78%, within the established by law. According to Normative Instruction No. 11 of 2000, the amount of reducing sugars for floral honey is at least 65% honey, and in melate honey, minimum 60% honey (BRASIL, 2000b).

A value similar to that found in the research was found by Aroucha (2008) when carrying out the work to study the quality of honeys produced by the incubators of Iagram and sold in the municipality of Mossoró / RN, in which he obtained values ranging from 66.97 to 75,0%. Silva et al. (2003), found values that varied from 73.52 and 80.26%.

For total sugar, the value found was 83.32%. Very close values were also observed by Silva et al. (2003), in which they obtained in their research values that varied from 77.15% to

81.50%.

When working with honey, it is common to find variations in its physicochemical composition, considering that various factors interfere with its quality, such as climatic conditions, maturation stage, bee species, processing and storage, in addition to the type of flowering (Silva et al., 2004).

Chemical and physicochemical stability

The variance analysis of the chemical and physicochemical data showed significant interactions ($P \leq 0.05$) among the treatments (formulations F1, F2 and F3) and the storage time (0, 30, 60, 90 and 120 days) for pH, soluble solids (SS), ascorbic acid, total phenolics and Chroma. Therefore for these data, it was done a separate regression analysis for each treatment.

About the data of total acidity, total sugars, reducing sugars total carotenoids, soluble brown pigments, L* and Hue there were not detected significant interaction ($P > 0.05$) between formulations and storage time. The differences among formulations were studied by the mean test (Tukey) (Table 2) and the evaluation of the parameters' behavior with time and storage, by regression analysis.

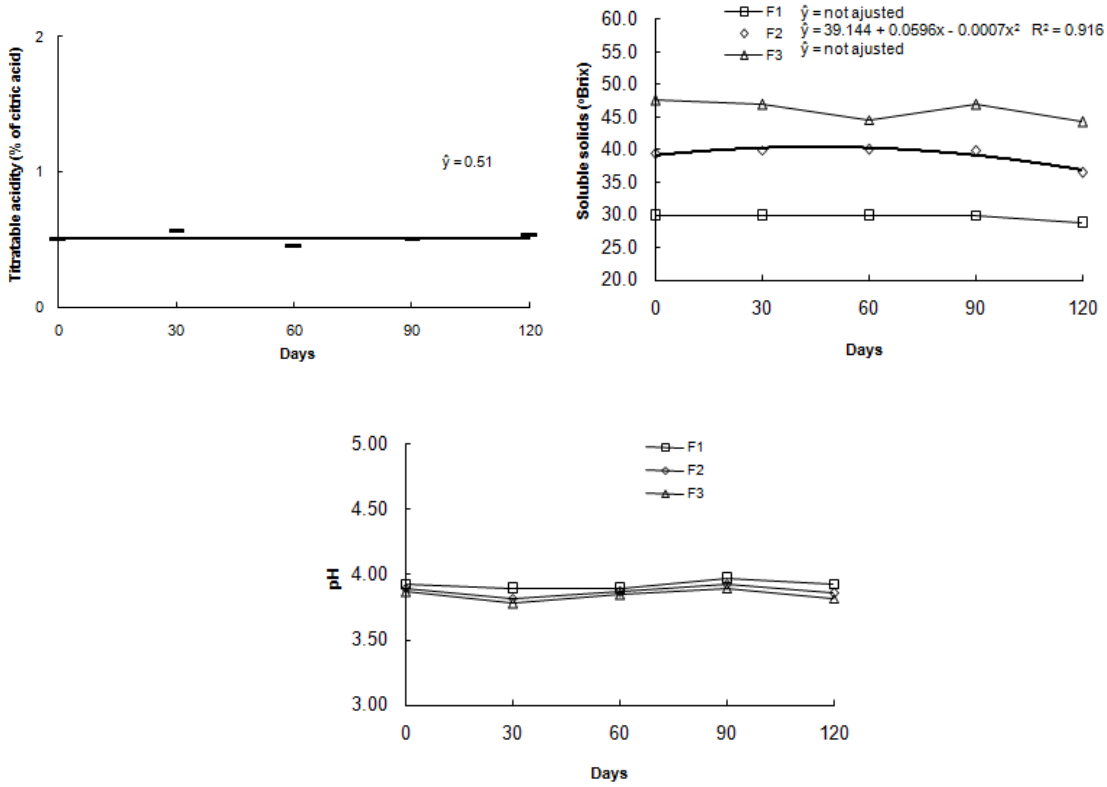
Table 2 - Comparison of means for total acidity, total sugar (TS) and reducing sugars (RS), carotenoids, soluble brow pigments, Luminosity (L*) and Hue for beverages formulated with cashew apple and honey

Formulations	Titratable acidity (% citric acid)	TS (%)	RS (%)	Carotenoids (μg.100 mL^{-1})	Soluble brown pigments (Abs. 420 nm)	L*	Hue
F1	0.55^{a}	30.88^{c}	29.60^{c}	29.48^{a}	0.26^{b}	58.66^{a}	84.69^{a}
F2	0.51^{a}	42.36^{b}	39.80^{b}	21.78^{b}	0.33^{ab}	53.27^{b}	83.43^{ab}
F3	0.47^{b}	50.63^{a}	47.45^{a}	16.74^{b}	0.40^{a}	46.22^{c}	81.97^{b}

Means in columns with different letters are significantly different ($P > 0.05$) by the Tukey test.
Source: Authors (2020).

Although presenting significant variation with storage time ($P \leq 0.05$), the pH was not possible to adjust the data to none of the models tested (Figure 2).

Figure 2 - pH and soluble solids of beverages F1 (75% of cashew apple pulp and 25% of honey), F2 (60% of cashew apple pulp and 40% of honey) and F3 (50% of cashew apple pulp and 50% of honey) and mean of titratable acidity of three beverages formulated with cashew apple pulp and honey during 120 days storage at room temperature (25±2°C).



Source: Authors (2020).

A change in the pH values showed that they were affected by the amounts of cashew apple and honey used in the formulations and storage time. Similar results were obtained by Freitas et al. (2006) studying the storage stability of acerola juice and by Branco et al. (2007) in a study with a blend of orange and carrot where it is suggested that the oxidation of organic acids by the storage time may explain the loss of acidity and increase of pH.

The statistical analysis of the data obtained for soluble solids during storage time showed significant differences ($P \leq 0.05$) adjusting to a quadratic model for the formulation F2 and not adjustable to the tested formulations F1 and F3 (Figure 2).

The soluble solids content showed no change up to 90 days of storage for all formulations, following a decrease after that period. Silva et al. (2008) also observed reducing soluble solids' values during cashew apple nectar storage sweetened with honey.

Total acidity was similar among the formulations. The formulations F1 and F2 were

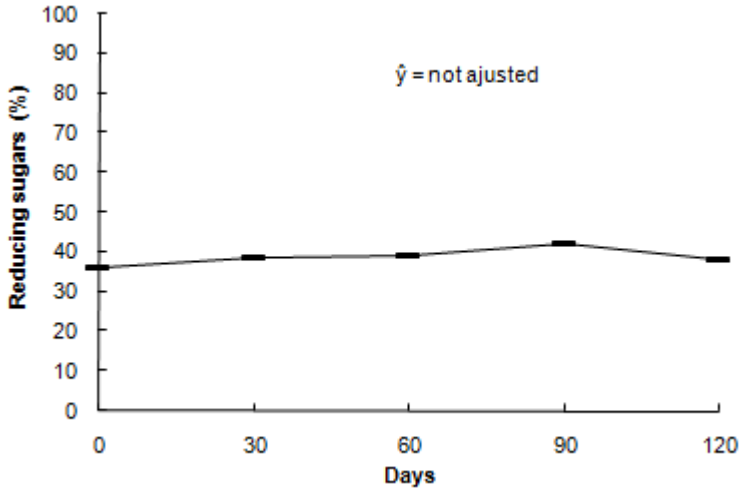
statistically equal by the Tukey test (P > 0.05), whereas the formulation F3 differed from the other two formulations (Table 2). Despite the significant interaction among the formulations and the storage time for the total acidity, it was impossible to adjust the data, represented by the means in each storage time (Figure 2). A decrease was observed in the acidity for up to 60 days. This initial loss may be due to the oxidation of the organic acids present.

The total sugar content presented significant variation with the storage time (P ≤ 0.05) but did not adjust to the tested models. The data were represented by the means in each storage time (Figure 3).

The data for reducing sugars showed a significant difference with storage time (Figure 3), but they did not adjust to the models tested. However, it was noted an increase in this parameter with storage time.

Sousa et al. (2010), working with mixed fruit nectars, observed a similar increase attributed to the non-reducing sugars hydrolysis used in the nectars' standardizations.

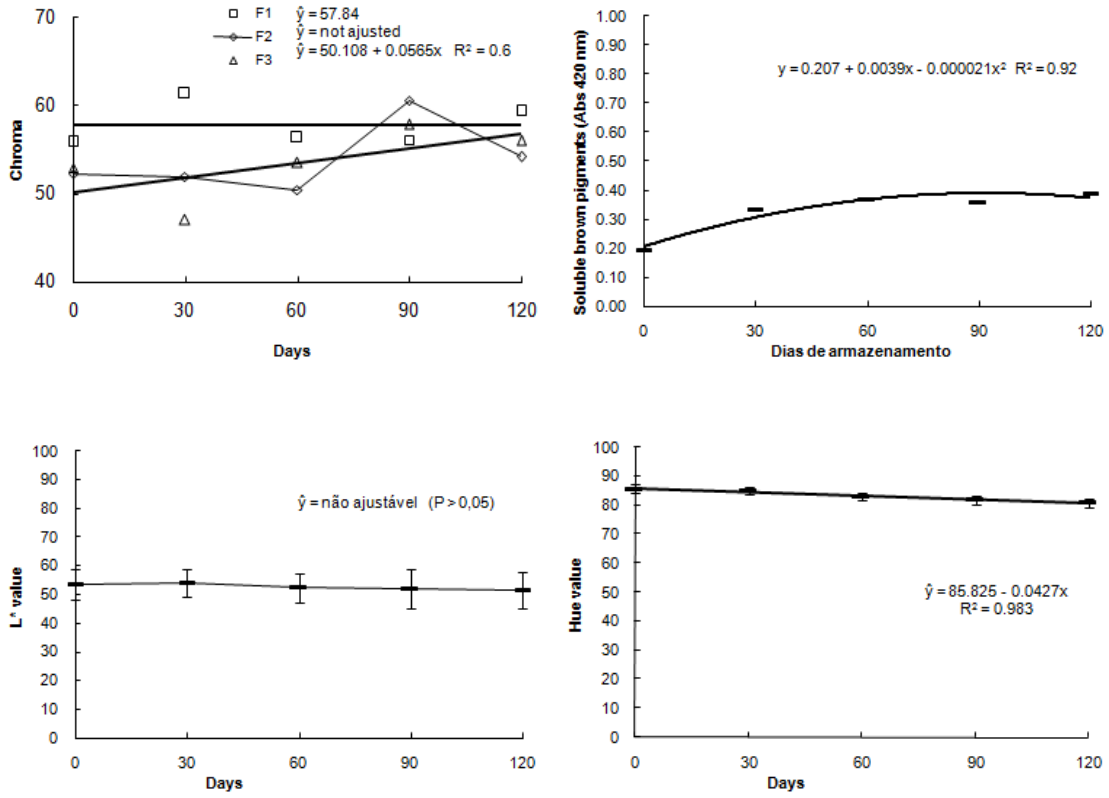
Figure 3 – Mean of beverages F1 (75% of cashew apple pulp and 25% of honey), F2 (60% of cashew apple pulp and 40% of honey), and F3 (50% of cashew apple pulp and 50% of honey) of total and reducing sugars during 120 days storage at room temperature (25±2°C).



Source: Authors (2020).

The brown pigments increased with time, showing a quadratic behavior (Figure 4), varying from 0.193 (time 0) to 0.386 (120 days), indicating darkening of the drinks.

Figure 4 - Chroma of beverages F1 (75% of cashew apple pulp and 25% of honey), F2 (60% of cashew apple pulp and 40% of honey), and F3 (50% of cashew apple pulp and 50% of honey) and mean of soluble brown pigments, L* and Hue of three beverages formulated with cashew apple pulp and honey during 120 days storage at room temperature (25±2°C).



Source: Authors (2020).

The water-soluble pigments of the formulation F2 did not differ statistically ($P > 0.05$) from the formulation F1 and F3. However, the last ones differed among them ($P \leq 0.05$). The F3 showed a higher value, 0.40; inferring that the proportion of honey added influenced the beverage color since this formulation presents a higher amount of honey.

The non-enzymatic browning can lead to loss of quality of the food by affecting its appearance due to new compounds such as 5-hydroxymethylfurfural (HMF) (Maia et al., 2007; Zhu et al., 2009). Carvalho et al. (2007) detected browning of a beverage prepared using green coconut water and cashew apple juice during storage.

Regarding color, the luminosity (L*) value varies from 0 (black) to 100 (white), being an indicator of browning. The L* differed statistically by the Tukey test among the formulations ($P \leq 0.05$). Therefore it is evident that the formulation F1, which contained a smaller amount

of honey, only 25% presented the higher mean of the formulations. The opposite happened with the formulation F3 (Table2). In this way, it is possible to correlate the L values found with brown pigments since the formulation F1 presented the smallest mean for brown pigments and greatest L* value. During the storage, the L* value showed a significant difference ($P \leq 0.05$); however, it was impossible to adjust the data to any model (Figure 4).

The initial increase of the L value can be caused by the carotenoid structure's thermic destruction furnishing a lighter color. However, because of the storage, other compounds' appearance, resulting mainly from the non-enzymatic browning (Remacha et al., 1992) or by the precipitation of pigments (Sistrunk and Cash, 1974) can be responsible for the browning of the product.

Hue is the state that characterizes the color tonality, allowing to differentiate the colors. The value for Hue did not present a significant difference between the formulations F2 and the formulations F2, F1, and F3 through the mean test ($P > 0.05$), but the formulations F1 and F3 differed among then ($P \leq 0.05$). The higher mean found was for formulation F1 followed by F2 and F3 (Table 2).

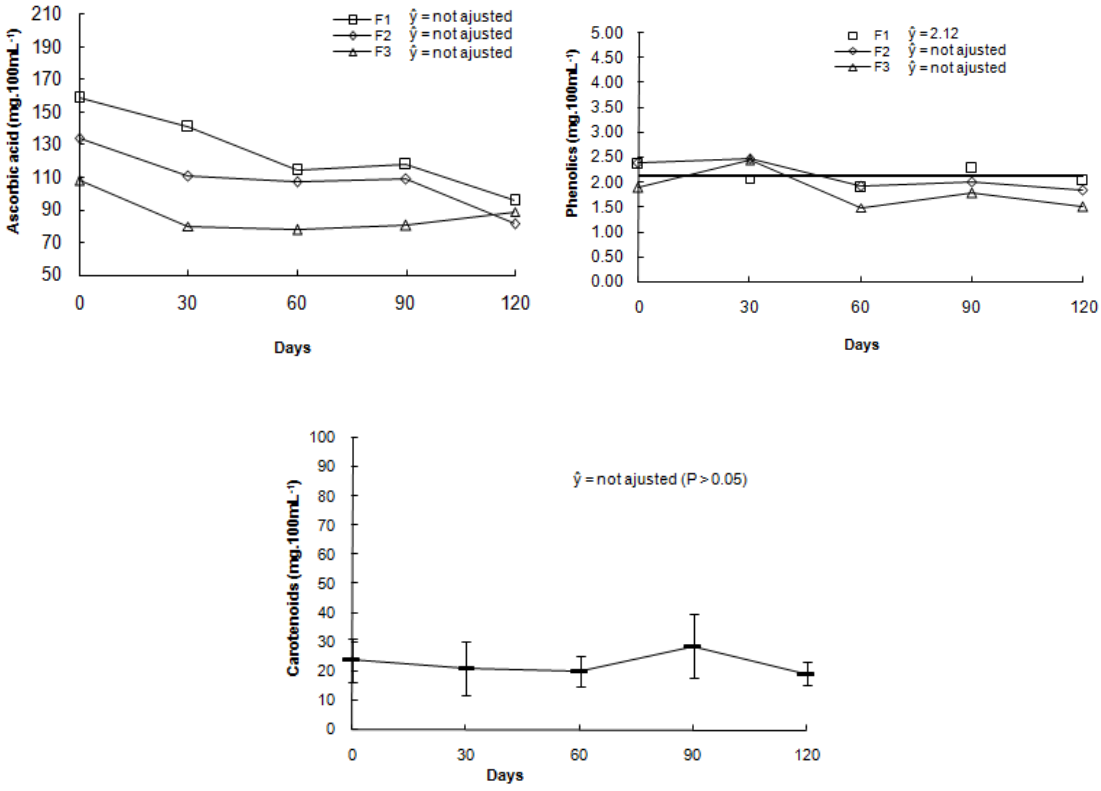
Adjusting a linear model, a decrease in the Hue values with storage (Figure 4) varied from 85.8 to 80.9. The values for the Hue angle suggest a small change in the yellowish color during the storage period.

The chroma represents the color saturation being the data that characterize the amount of color indicating the proportions mixed with white, black or grey. Therefore higher being the chroma greater is the number of pigments.

The chroma values for the formulation F1 did not significantly differ during the storage time ($P > 0.05$). The formulation F3 adjusted into a linear model showing a small increase during storage, varying from 50.11 to 56.89. The formulation F2, despite the present variation with storage, adjusted to none of the models tested. The chroma decrease with storage time indicates a reduction in the characteristic juice color (Figure 4), associated with carotenoids' oxidation (Sarantopoulos et al., 2001).

The values for ascorbic acid presented significant differences during the storage period ($P \leq 0.05$); however, it was impossible to adjust the data in none of the models for the three formulations tested being represented by the mean of each storage time (Figure 5).

Figure 5 - Ascorbic acid and phenolics of beverages F1 (75% of cashew apple pulp and 25% of honey), F2 (60% of cashew apple pulp and 40% of honey) and F3 (50% of cashew apple pulp and 50% of honey) and mean of carotenoids of three beverages formulated with cashew apple pulp and honey during 120 days storage at room temperature (25±2°C).



Source: Authors (2020).

The major loss of ascorbic acid was for the formulation F1, showing a 39.8% decrease after 120 days of storage, followed by formulation F2 39.2% of loss and formulation F3 17.7%. These losses can be associated with the storage temperature, exposition of the beverages to the light through the glass containers, and oxidation reactions due to oxygen dissolved in the liquid and in the container's headspace (Carvalho, 2007).

The greater loss of ascorbic acid in the formulation F1 may be explained by the higher amount of cashew apple pulp added. A smaller proportion of honey being observed as the opposite for the formulation F3.

Silva et al. (2008) studied the storage stability of cashew apple nectar sweetened with honey and observed a reduction of 93.3% of the ascorbic acid during 180 days of storage.

Knowing that the recommended daily allowance (RDA) for adults of vitamin C is 45 mg (Brasil, 2005, Fao/Who, 2001), at the end of 120 days, a portion of 200 mL of diluted drink

provides 141.8%, 90,6%, and 79.2% of the RDA, respectively, for F1, F2, and F3, featuring drinks as an excellent source of vitamin C.

The formulation F2 did not present the Tukey test difference regarding the carotenoids, whereas the formulation F1 differed from the two others and showed a higher mean (29.48 mg.100mL-1) (Table 2). For the carotenoid data, it was impossible to adjust the data to none of the statistic tested models despite the significant difference in storage time being their values represented by the mean of formulations for each time (Figure 5).

It was detected a trend of decreasing in carotenoids content with storage time. The loss of carotenoids can be associated with light exposure through glass bottles. One of the major causes of color loss during storage is carotenoid oxidation, accelerated by light, temperature, and metallic catalyzers (Sarantopoulos et al., 2001).

The total phenolics presented significant differences among the formulations F2 and F3. Still, it was not possible to adjust the data to the tested models being represented by the mean of formulations for each time. The formulation F1 showed a constant value for total phenolics, with an average of 2.12mg of AGE.mL-1 (Figure 5).

4. Conclusions

The physical and chemical parameters presented small variations along the storage period for the three developed formulations.

Ascorbic acid was the parameter that showed the greatest loss during storage. However, the beverages can be considered a good source of this vitamin.

The preparation of cashew apple pulp beverages with honey to dilute is viable, feasible within the processing used, becoming an alternative use and value to the cashew apple and honey.

Despite the verification of the product's stability to chemical and physicochemical parameters, further studies should be carried out to assess the products' microbiological stability and sensory profile.

Acknowledgments

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