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Faria FREITAS, Mírian Luisa; Borges de Lima DUTRA, Mariana; André BOLINI, Helena
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Development of pitanga nectar with different sweeteners by sensory analysis: Ideal pulp dilution, ideal sweetness, and sweetness equivalence

Mírian Luisa Faria FREITAS^{1*}, Mariana Borges de Lima DUTRA¹, Helena Maria André BOLINI¹

Abstract

The objective of this study was to develop pitanga nectar formulations in which sucrose was replaced with different sweeteners. Consumer tests were conducted with 50 fruit juice consumers, and a just-about-right scale was used to determine the ideal pulp dilution and ideal sweetness with sucrose. Furthermore, the adequate concentrations of six sweeteners were determined to obtain the equivalent sweetness of sucrose using relative to these concentrations the magnitude estimation model with 19 selected assessors. The ideal dilution test resulted in 25% pulp, and the ideal sweetness test, 10% sucrose. Sweetener concentrations to replace sucrose were 0.0160%, 0.0541%, 0.1000%, 0.0999%, 0.0017%, and 0.0360%, respectively, for sucralose, aspartame, stevia 40% rebaudioside A, stevia 95% rebaudioside A, neotame, and a 2:1 cyclamate/saccharin blend. These results can be used to prepare pitanga nectar with different sweeteners and obtain the same sweetness intensity in less caloric products than that of nectar prepared with sucrose.

Keywords: tropical fruit; beverage; just-about-right scale; magnitude estimation.

1 Introduction

Many Brazilian fruits have great market potential; therefore, studying processes that increase their value or their application in food products is of great interest. Some types of juice, such as orange, apple, grape, pineapple, and tomato are well established in developed countries. Other kinds of juice, especially tropical fruit juices, have drawn attention due to their nutritional value and functional characteristics (Ongaratto & Viotto, 2009).

Pitanga (*Eugenia uniflora* L.), also known as “surinam cherry” or “Brazilian cherry”, is a tropical fruit which belongs to the Myrtaceae family (Bezerra et al., 2000; Gomes, 1975). It contains high carotenoid levels (32% of the carotenoid total is lycopene) and significant amounts of vitamin A and vitamin C (Lima et al., 2002).

Refined sugars, syrups, or artificial sweeteners are used to sweeten fruit juice and nectar. However, it is important to consider that the increase in obesity has been partly attributed to an overall increase in refined sugar consumption, such as sucrose and fructose. Due to their intense levels of sweetness, artificial sweeteners can provide the proper sweet taste without adding calories to foods (Mahar & Duizer, 2007).

Due to the large Brazilian production, Pitanga can also be used in the food industry. In Brazil, the largest commercial scale planted, which is also the largest in Latin America is located in the state of Pernambuco, and the state annual production is estimated to be 1,300-1,700 ton/year (Silva, 2006). In the Brazilian food industry, pitanga has been used mainly for juice production, which shows good economic potential due to its high concentration of vitamins and minerals (Lima et al., 2002).

In a study on Brazilian exotic fruit juices consumption, Vidigal et al. (2011) found that 40.6% of the consumers consider

the healthy food or the presence of beneficial compounds as the most important factors for the consumption of food products and that 49.1% of consumers consider pleasant taste as the most important motivation for consumption. According to the latest market research conducted by the Brazilian Association of Industries of Soft Drinks and Non-Alcoholic Beverages (ABIR), the consumption of juices and nectars in Brazil grew 14.9% between 2009 and 2010, reaching 543 million liters in 2010. Between 2005 and 2010 this growth was 72% (Associação Brasileira das Indústrias de Refrigerantes e de Bebidas não Alcoólicas, 2014).

Therefore, there is a great potential market for pitanga nectar. In addition, the substitution of sweeteners for sucrose meets the needs of consumers who cannot eat sugar due to metabolic disorders or those seeking a less caloric beverage. In spite of some available studies, there is still a lack of data regarding the processing of tropical fruits, including pitanga (Ongaratto & Viotto, 2009). Accordingly, the objective of this study was to develop pitanga nectar formulations in which sucrose was replaced with different sweeteners.

2 Materials and methods

2.1 Materials

The pitanga nectar samples were prepared with unsweetened frozen pulp (Ricaeli, Cabreúva, Brazil); subsequently, different sweeteners were added. The pulp was previously pasteurized at 85 °C for 25-42 seconds. The sweeteners evaluated were: sucrose (União, São Paulo, Brazil), sucralose (Tovani-Benzaquem, São Paulo, Brazil), aspartame (All Chemistry do Brasil, São Paulo, Brazil), stevia 40% rebaudioside A (Clariant, Suzano, Brasil),

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¹Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas – UNICAMP, Campinas, SP, Brazil, e-mail: mirianlfreitas@yahoo.com.br

*Corresponding author

stevia 95% rebaudioside A (Tovani-Benzaquem, São Paulo, Brazil), neotame (Sweetmix, Sorocaba, Brazil), and (2:1) a cyclamate (Sweetmix, Sorocaba, Brazil) and saccharin (Pharma Nostra, Rio de Janeiro, Brazil) blend.

2.2 Methods

Pulp characterization

Analysis of soluble solids in Brix was performed using a Carl ZEISS refractometer (844 976), as established by the AOAC Official Method 932.12 (Association of Official Analytical Chemists, 1995). The samples were filtered through cotton before the readings. The pH was measured using a potentiometer (Tecnopon MPA 210), as established by the AOAC Official Method 981.12 (Association of Official Analytical Chemists, 1995). Titratable acidity was assessed by titration with NaOH, as established by the AOAC Official Method 942.15 (Association of Official Analytical Chemists, 1995), to an end point of pH of 8.1. Five grams of the sample were used to facilitate identifying the end point. Color was measured using the Hunter system, with a ColorQuest II colorimeter (HunterLab). The parameters L^* (lightness), a^* (color between green and red) and b^* (color between blue and yellow) were measured. The ascorbic acid concentration was determined by titration with 2,6-dichlorophenolindophenol, as established by the AOAC Official Method 43.065 (Association of Official Analytical Chemists, 1984). This method was modified by Benassi & Antunes (1988), who replaced the organic solvent metaphosphoric acid with oxalic acid. All tests were performed in triplicate.

The microbiological characterization was based on testing for *Salmonella*, as established by the AOAC Official Method 031001 (Association of official analytical chemists, 2010) and by the horizontal method for detection of *Salmonella* spp., established by ISO 6579 (International Organization for Standardization, 2002); coliform bacteria by the horizontal method for the detection and enumeration of coliforms, as established by ISO 4831 (International Organization for Standardization, 2006); Thermotolerant coliform by the horizontal method for the detection and enumeration of presumptive *Escherichia coli*, as established by ISO 7251 (International Organization for Standardization, 2005); and *Alicyclobacillus* by the method on the detection of Taint Producing *Alicyclobacillus* in Fruit Juices, as established by IFU 12/2007 (International Federation of Fruit Juice Producers, 2007). The microbiological analyses were performed at the Center for Science and Food Quality of the Institute of Food Technology, (Campinas, São Paulo, Brazil).

Sample preparation

The pitanga nectar was prepared at the Laboratory of Sensory Analysis, Department of Food and Nutrition of the University of Campinas, on the day before each test. Frozen pulp, drinking water, and sweeteners were used. Each sample was sweetened with the respective sweetener and homogenized for about two minutes. The samples were stored in bottles in a refrigerator (about 10 °C).

The samples were presented in a balanced block design (Macfie et al., 1989) in plastic disposable cups coded with a three-digit number. The tests were conducted in individual booths at the Sensory Analysis Laboratory.

Ideal pulp dilution determination

The first step in the development of the pitanga nectar was to determine the ideal pulp dilution. The samples were prepared with different pulp concentrations: 25%, 31%, 37.5%, 44%, and 50%, and sweetened with 10% sucrose.

An acceptance test was conducted with 50 fruit juice consumers. A 9-cm unstructured just-about-right scale was used. It ranged from “extremely less concentrated than the ideal” to “extremely more concentrated than the ideal”. The middle point of the scale corresponded to “ideal” (Meilgaard et al., 1999).

The results were analyzed by simple linear regression analysis between the hedonic value and the pulp concentration, as suggested by Vickers (1988).

Ideal sweetness determination

After obtaining the ideal dilution, a test to determine the ideal sweetness of the pitanga nectar was conducted. The samples were prepared with the ideal dilution with different sucrose concentrations: 5%, 7.5%, 10%, 12.5%, and 15%.

The test was conducted with 50 fruit juice consumers, as previously described (Meilgaard et al., 1999; Vickers, 1988).

Group selection

The selection of the sensory panel members for the sweetness equivalence test was performed using the Wald sequential analysis (Amerine et al., 1965). The triangular difference tests were used to select candidates with good sensory discrimination ability.

Two nectar samples were used at the ideal pulp concentration (with 3.5% and 5.0% sucrose). A paired comparison test was conducted with 16 tasters to prove that the samples were different. A significant difference was detected with 90% confidence.

The following parameters were used in the sequential analysis to select the candidates: $\rho_0 = 0.33$ (maximum acceptable inability), $\rho_1 = 0.66$ (minimum acceptable ability), $\alpha = 0.05$ (probability of accepting a candidate without sensory acuity), and $\beta = 0.05$ (probability of rejecting a candidate with sensory acuity) (Moraes & Bolini, 2010; Augusto et al., 2005). They defined, in a graph, regions of acceptance, rejection, and an intermediate region in which the taster should proceed with testing.

Equi-sweetness determination

Measurements of the relative sweetness of the sweeteners were performed according to the method of magnitude

estimation (Stone & Oliver, 1969), which provides a direct quantitative measure of subjective sweetness intensity.

The assessors received a reference sample with sweetness intensity designated by the arbitrary value 100, followed by several samples with higher or lower sweetness intensity. They were asked to estimate the sweetness intensity of the samples and compare them to the reference sample. For example, if the sample was twice as much sweeter, it should receive the score of 200; if it was 50 percent sweeter, it should receive the score of 50, and so on. The score 0 could not be assigned to the samples.

The concentrations of each sweetener were determined in aqueous solutions, as described by Bolini-Cardello et al. (1999) and used by Cardoso & Bolini (2007), Moraes & Bolini (2010) and Cadena & Bolini (2012), as shown in Table 1.

The statistical software program SAS (Statistical Analysis System Institute, 2012) was used for data analysis. Estimated sweetness magnitude values were converted to logarithmic values and expressed by geometric means. Sensory responses to the sweeteners versus concentration curves corresponded to a Power Function with the following characteristics: $S=a \cdot C^n$, where S is the perceived sensation, C is the stimulus concentration, a is the value of y at the intercept antilog, and n is the slope coefficient obtained (Moskowitz, 1974).

This research project was submitted to and approved by the Research Ethics Committee from the University of Campinas, CEP n° 1264/2011. Moreover, a Term of Consent containing information about the research was prepared and presented to the tasters.

3 Results and discussion

3.1 Pulp characterization

The results of the physicochemical analyses can be seen in Table 2. According to the Brazilian requirements for fruit pulp, pitanga pulp must have soluble solids content greater than 6 °Brix, pH 2.5 to 3.4, and titratable acidity greater than 0.92% citric acid (Brasil, 2000). Therefore, it is the pitanga pulp used met the Identity and Quality Standards requirements.

Mélo et al. (1999) characterized manually mashed pitanga pulp and found similar results to those found in the present study: 10.80 °Brix for soluble solids, pH 2.60, and 2.04% citric acid by titratable acidity. The amount of ascorbic acid obtained

by Mélo et al. (1999) was 95.00 mg/100 g, while 11.187 mg/100 g of pitanga pulp was found in the present study. The variation in ascorbic acid content in fruits can be explained by several factors that affect its synthesis and retention. These include variety and environmental factors, especially insolation and cultivation (Dib Taxi et al., 2003), and also processing and storage conditions (Lopes et al., 2005). Pasteurization causes low level-loss of ascorbic acid due to the reduced time under high temperature; but the freezing process and storage at freezing temperatures usually cause retention of ascorbic acid and pigments in food (Rodríguez-Amaya, 1999; Aquino et al., 2011). As reported by Aquino et al. (2011), for example, no significant differences ($p > 0.05$) were observed in the levels of ascorbic acid in frozen acerola fruits stored for 60 days.

Chaves et al. (2013), when characterizing purple pitanga pulp, obtained soluble solid concentration of 8.29 °Brix, pH of 3.11, 1.62% citric acid by titratable acidity and color parameters of 31.48, 19.55 and 7.17, respectively, for L^* , a^* and b^* . This b^* value is than that found in the present study, which indicates that the color of the sample corresponds to blue (blue hue).

The microbiological characterization results are in accordance with the Brazilian legislation requirements for fruit pulp (Brasil, 2001), which establishes that the coliform count at 45 °C should not exceed the limit of 10^2 per gram, and *Salmonella* should be absent in 25 g. However, the Brazilian legislation has not established a limit for *Alicyclobacillus*, but this analysis was carried out anyway because it deals with a non-pathogenic thermoacidophilic spore-forming bacterium, one that can germinate and grow in acidic fruits and contaminate juices and nectars. Thus, the pulp tested proved a safe product, and it is adequate for sensory analysis.

According to Lopes et al. (2005), frozen storage of pitanga pulp is the most viable alternative from the product quality point of view.

3.2 Ideal pulp dilution determination

Data of the pulp concentrations were analyzed by linear regression according to the means of the scores assigned to each concentration (Figure 1). According to the linear equation, the ideal pitanga pulp concentration was 24% (Figure 1). However, according to the Brazilian legislation (Brasil, 2003), the minimum amount of pulp for pitanga nectar production is 25%. Thus, the pitanga pulp concentration was determined as 25% for further analyses.

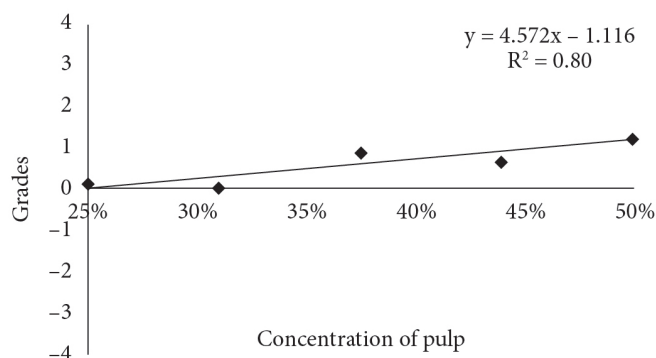
Table 1. Sweetener concentrations to determination of equi-sweet concentrations.

Sweetener	Concentration of sweeteners to determine equi-sweet to 10% of sucrose ^a				
Sucrose	3.9100	6.2500	10.0000	16.0000	25.6000
Sucralose	0.0063	0.0100	0.0160	0.0256	0.0410
Aspartame	0.0200	0.0340	0.0550	0.0880	0.1408
Stevia 40% rebaudioside A	0.0391	0.0625	0.1000	0.1600	0.2560
Stevia 95% rebaudioside A	0.0391	0.0625	0.1000	0.1600	0.2560
Neotame	0.0007	0.0010	0.0017	0.0027	0.0041
2:1 Cyclamate/saccharin blend	0.0141	0.0225	0.0360	0.0576	0.0923

^a Concentrations in percentage (w/v).

Table 2. Physicochemical characterization of pitanga pulp.

Determination	Means
Solid soluble (°Brix)	6.45 °Brix
pH	3.12
Titrateable acidity (% citric acid)	1.19% citric acid
Color	L* a* b*
	40.39 19.84 19.54
Ascorbic acid	11.187 mg/100 g of pulp

**Figure 1.** Pitanga pulp concentration versus the nectar scores attributed by the consumers.

Although pitanga pulp manufacturers recommend beverage preparation with 33% pulp, the ideal pulp dilution test showed 25% pulp as the most acceptable concentration in the range from 25% to 50%. The higher dilution obtained may result from the fact that this fruit has strong flavor and an acidic taste (Bezerra et al., 2000), and therefore it needs further dilution to provide a more pleasant flavor. This result allows industries to cut costs when manufacturing pitanga nectar.

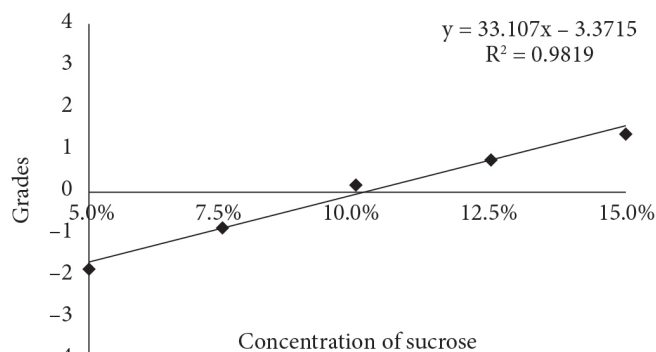
3.3 Ideal sweetness determination

This variable was assessed using the same procedure described previously to determine the ideal pulp dilution. Linear regression analysis (Figure 2) shows that the ideal estimated sweetness value was 10% sucrose.

Ideal sweetness and sweetness potency vary in different products. In studies to determine ideal sweetness of juice and nectar, Cardoso & Bolini (2007), Cadena & Bolini (2012), Marcellini et al. (2005) and De Marchi et al. (2009) observed, respectively, 10% sucrose in peach nectar, 7% sucrose in mango nectar, 8.5% sucrose in pineapple juice, and 10% sucrose in passion fruit juice. As for coffee and tea, Moraes & Bolini (2009) found 9.5% and 12.5% sucrose, respectively, for instant coffee and roasted ground coffee, and Cardoso et al. (2004) found 8.3% sucrose for mate tea at 6 °C and 45 °C. With regard to yogurt, Reis et al. (2011) obtained 11.5% sucrose for strawberry yogurt.

3.4 Group selection

According to the number of triangular tests and the cumulative number of correct answers, 19 assessors were

**Figure 2.** Sucrose concentration versus nectar scores attributed by the consumers.

selected. Some candidates were selected after choosing the correct sample in seven tests out of nine. Others candidates were selected after choosing the correct sample in eight tests out of twelve. All assessors selected were within the acceptance region of the graph.

3.5 Equi-sweetness determination

The selected assessors evaluated all sweeteners at different concentrations using the magnitude scale. Table 3 shows the angular coefficient, Y-intercept, determination coefficient, and Power Function of each sweetener.

Values higher than 0.9 were found for all of the sweeteners with respect to the coefficient of determination in the method for estimating magnitude (Table 3). The lowest value (0.9726) was found for the 2:1 cyclamate/saccharin blend. Cardoso & Bolini (2007) and Moraes & Bolini (2010) found similar coefficients of determination (0.9600 and 0.9640), respectively, for stevia in peach nectar and for the 2:1 cyclamate/saccharin blend in instant coffee. According to Cardoso & Bolini (2007), a possible explanation is that these sweeteners have a bitter taste, especially in high concentrations. This characteristic probably influenced the perception of sweetness, as well as the linearity.

Figure 3 shows the relationship between perceived sweetness and the sweetness power of each sweetener concentration on a logarithmic scale. It is important to highlight that in order to achieve equi-sweetness, it is necessary to use larger amounts of the two kinds of stevia than neotame, for example.

Table 4 shows the equivalent concentration and the sweetness potency of each sweetener in relation to the 10% sucrose pitanga nectar. Neotame was 5882 times sweeter, and the two kinds of stevia were 100 times sweeter than 10% sucrose in pitanga nectar.

Sucralose showed sweetener potency 625 times higher than that of 10% sucrose in pitanga nectar (Table 4). Cardoso & Bolini (2007) and Cadena & Bolini (2012) found similar values (629 and 627), respectively, for 10% sucrose in peach nectar and 7% sucrose in mango nectar, while Marcelini et al. (2005) obtained a lower value (494) for 8.5% sucrose in pineapple juice. In addition, Moraes & Bolini (2010) found similar results (636 and 599), respectively, for 9.5% sucrose in instant coffee and

Table 3. Angular coefficient, Y-intercept, coefficient of determination (R^2), and Power Function of each sweetener tested.

Sweetener	Angular Coefficient	Y-intercept	R^2	Power Function
Sucrose	1.1868	-1.1869	0.9806	$P = 0.0650 \cdot C^{1.1868}$
Sucralose	1.0056	1.8052	0.9988	$P = 63.8557 \cdot C^{1.0056}$
Aspartame	1.0213	1.2939	0.9974	$P = 19.6698 \cdot C^{1.0213}$
Stevia 40% rebaudioside A	0.9078	0.9078	0.9754	$P = 8.0872 \cdot C^{0.9078}$
Stevia 95% rebaudioside A	0.8032	0.8032	0.9743	$P = 6.3562 \cdot C^{0.8032}$
Neotame	1.0891	3.0234	0.9949	$P = 1055.3585 \cdot C^{1.0891}$
2:1 Cyclamate/saccharin blend	1.2167	1.7561	0.9726	$P = 57.0296 \cdot C^{1.2167}$

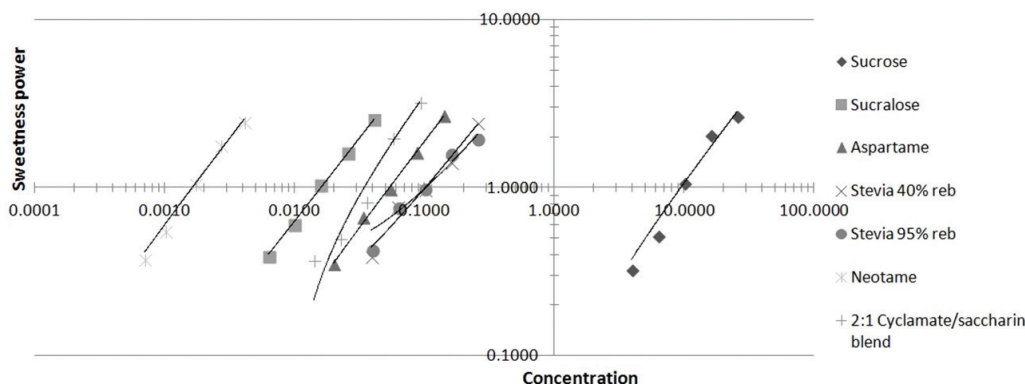


Figure 3. Relationship between perceived sweetness and sweetener concentrations in pitanga nectar.

Table 4. Equivalent concentration and sweetness potency to sucrose 10%.

Sweetener	Concentration equivalent to sucrose 10%	Sweetness potency at 10%
Sucralose	0.0160%	625
Aspartame	0.0541%	185
Stevia 40% rebaudioside A	0.1000%	100
Stevia 95% rebaudioside A	0.0999%	100
Neotame	0.0017%	5882
2:1 Cyclamate/saccharin blend	0.0360%	278

12.5% sucrose in roasted ground coffee. Cardoso et al. (2004) obtained a similar result (678) for 8.3% sucrose in mate tea at 45 °C and a lower value (554) for 8.3% sucrose in mate tea at 6 °C. Moreover, Reis et al. (2011) reported a lower value (388) for 11.5% sucrose in strawberry yogurt.

Aspartame had sweetener potency 185 times higher than 10% sucrose in pitanga nectar (Table 4). Cardoso & Bolini (2007) found similar results (185) for 10% sucrose in peach nectar, while Marcellini et al. (2005) observed a lower value (144) for 8.5% sucrose in pineapple juice., Moraes & Bolini (2010) also obtained similar results (188 and 173), respectively, for 9.5% sucrose in instant coffee and 12.5% sucrose in roasted ground coffee. Cardoso et al. (2004) found a similar value (163) for 8.3% sucrose in mate tea at 45 °C and a higher value (277) for 8.3% sucrose in mate tea at 6 °C. Furthermore, Reis et al. (2011)

obtained a similar result (160) for 11.5% sucrose in strawberry yogurt.

Both stevia 95% rebaudioside A and stevia 40% rebaudioside A had sweetener potency 100 times higher than that of 10% sucrose in pitanga nectar. Stevia consists of stevioside and rebaudioside, among other molecules, and rebaudioside had greater sweetness than stevioside (Barriocanal et al., 2008). Despite the difference in rebaudioside in the two kinds of stevia, they showed similar sweetener potency. This result suggests that the bitterness of the sweeteners should have masked the sweet taste. Therefore, the two kinds of stevia obtained the lowest sweetness among all sweeteners studied (Table 4). Cardoso & Bolini (2005) found similar results (101) for 10% sucrose in peach nectar; Cadena & Bolini (2012) found a higher value (134) for 7% sucrose in mango nectar, while Marcelini et al. (2005) obtained lower sweetness potency (63) for 8.5% sucrose in pineapple juice. Moraes & Bolini (2010) observed similar results (101) for 9.5% sucrose in instant coffee and a lower value (75) for 12.5% sucrose in roasted ground coffee. Additionally, Cardoso et al. (2004) found similar results (118) for 8.3% sucrose in mate tea at 45 °C and a lower value (83) for 8.3% sucrose in mate tea at 6 °C.

On the other hand, neotame had the highest sweetness level among all sweeteners, and it was 5882 times sweeter than 10% sucrose in pitanga nectar (Table 4). Cadena & Bolini (2012), when studying sweeteners in 7% sucrose mango nectar, also found a high value (6026) for neotame sweetness.

The 2:1 cyclamate/saccharin blend had sweetener potency 278 times higher than that of 10% sucrose in pitanga nectar

(Table 4). Cardoso & Bolini (2007) reported similar results (280) for 10% sucrose in peach nectar. In contrast, Marcellini et al. (2005) found a lower value (220) for 8.5% sucrose in pineapple juice. Furthermore, Moraes & Bolini (2010) reported similar results (280) for 9.5% sucrose in instant coffee and a lower value (215) for 12.5% sucrose in roasted ground coffee. However, Cardoso et al. (2004) found similar results (272) for 8.3% sucrose in mate tea at 45 °C and a higher value (332) for 8.3% sucrose in mate tea at 6 °C.

Therefore, sweetness potency in different beverages available in literature, regarding pitanga nectar, ranged from 54% to 108% for sucralose (Reis et al., 2011; Cardoso et al., 2004), from 78% to 150% for aspartame (Marcellini et al., 2005; Cardoso et al., 2004), from 63% to 134% for stevia (Marcellini et al., 2005; Cadena & Bolini, 2012), from 77% to 119% for the 2:1 cyclamate/saccharin blend (Moraes & Bolini, 2010; Cardoso et al., 2004), and 102% for neotame (Cadena & Bolini, 2012).

It is worth noting that the equivalent sweetness levels found for high potency sweeteners are highly dependent on the product. They may vary in different products (Redlinger & Setser, 1987), and this must be taken into account during the development of new products.

4 Conclusion

Based on the acceptance tests using a just-about-right scale, it was found that pitanga nectar should consist of 25% pulp and 10% sucrose.

Various sweeteners were tested as sucrose replacements. The magnitude scale determined the amount of each sweetener required to achieve sucrose equi-sweetness. In addition, the sweetness potency of sucrose, aspartame, stevia 40% rebaudioside A, stevia 95% rebaudioside A, neotame, and the 2:1 cyclamate/saccharin blend, respectively, were 625, 185, 100, 100, 5882 and 278. Therefore, neotame had the highest sweetener potency and both kinds of stevia had the lowest potency.

Sensory analysis tests are essential for the development of a product that meets the needs of consumers. Therefore, it was possible to develop formulations with different sweeteners to produce low calorie pitanga nectar with good nutritional and functional properties.

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