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Multiple Time-Intensity Analysis and Parameters Affecting the Acceptance of Low-Calorie Acerola (*Malpighia emarginata* dc.) Nectar

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Received: June 17, 2020; **Published:** June 30, 2020

DOI: 10.31080/ecnu.2020.15.00849

Abstract

The aim of this study was to evaluate the gustatory stimuli sweet, bitter, sour and acerola flavor of acerola nectar using the time-intensity method and identify the parameters of the time-intensity curve affecting product acceptance. Sucrose, sucralose, neotame, and stevia extracts with 40%, 60%, 80% and 95% Rebaudioside A were used to sweeten the samples. Twelve assessors participated in the time-intensity analysis and a hundred and twenty consumers evaluated the overall impression. Sample sweetened with neotame showed greater intensity and duration for the sweet stimulus. With respect to the bitter stimulus, the samples containing different stevia extracts presented the highest intensity and stimulus duration, evidencing the bitter aftertaste. The time-intensity curves for the sour stimulus were very similar. The time-intensity curves for acerola flavor stimulus were similar too, but the sample sweetened with stevia extract 95% Rebaudioside A showed low-intensity stimulus, whereas samples containing sucrose and sucralose exhibited the greatest stimulus duration. The parameters total duration and area under the curve of the bitter stimulus negatively affected the acceptance of acerola nectar.

Keywords: *Tropical Fruit; Malpighia emarginata DC; Partial Least Squares Regression; Sensory Analysis*

Introduction

Acerola (*Malpighia emarginata* DC.) is a plant native to Central America and its global spread has started by the Spaniards during the colonization period. For its undoubted potential as a natural source of vitamin C and its great capacity for industrial use, acerola fruits have attracted the interest of fruit growers with economic importance in several regions of Brazil [1]. Mezadri., *et al.* [2] reported that the acerola fruits have high antioxidant activity due to ascorbic acid content and the presence of polyphenols, evidencing the fruit and its derivatives as potent antioxidants.

The consumption of processed fruit juices and nectars has increased in Brazil and in the world, motivated by several factors including lack of time to make juice using fresh fruits, practicality, replacement of carbonated beverages and concern in healthier food intake [3]. Faced with the need to replace sucrose by sweeteners, the interest on studying these substances has increased due to not only the health problems caused by sucrose ingestion, such as obesity, diabetes and dental caries, but because of beauty standards set by society [4]. According to Hunty, Gibson and Ashwell [5] strategies to reduce rates of overweight and obesity has focused on reducing caloric intake and increasing energy expenditure. The use of sweeteners as sucrose substitutes is a technology to reduce energy intake without loss of palatability.

Stevia rebaudiana Bertoni is native to Paraguay, where it has been used for over a century to sweeten beverages, known as Caá He-E that means sweet herb in Guarani. This plant has a class of compounds known as steviol glycosides, which are produced by the plant at high concentrations. The most important glycosides are stevioside, rebaudioside A, rebaudioside C and dulcoside A, and stevioside and rebaudioside A are present in higher concentrations [6]. In general, the of ratio rebaudioside A to stevioside is the accepted measure of sweetness quality. The predominance of stevioside confers the extract a characteristic bitter taste, while extracts containing higher concentrations of rebaudioside A are characterized by higher solubility, greater sweetness and less bitterness, with a higher market price [7].

Neotame exhibits a sweetness profile close to sucrose. As with other sweeteners, the sweetness potency of neotame depends on its concentration and on the food or beverage to be added [8]. Sucralose is manufactured from the selective replacement of three hydroxyl groups on the sucrose molecule by three chlorine atoms. The selective chlorination stabilizes sucralose, which prevents it from being degraded or metabolized, changing the sweetness intensity, providing an intensely sweet taste about 600 times sweeter than sucrose, without compromising the sweet taste profile [9,10].

The time-intensity analysis is a method used to study changes in intensity of sensory properties over time. Early attempts to build the time-intensity curves began by determining the mean intensity values at certain times [11,12]. This technique is important to provide information about consumer's behavior on the perception of flavors during food intake, and it is used to obtain a temporal profile of an attribute for a particular food [13]. It quantifies the perceived intensity of a single stimulus according to the elapsed time (speed, duration, and intensity) when evaluating a sample [14]. Although the time-intensity technique has been used for a long time to evaluate a single attribute, more than one attribute can be evaluated, thus it is known as multiple time-intensity analysis [15,16].

Meeting consumer needs is a priority of the market. In this regard, the acceptance of a product by consumers is considered as a trigger for subsequent purchases and, therefore, a factor contributing to the success of companies in the long term. Moreover, a panel of trained assessors evaluates the food sensory quality according to specific sensory descriptors. The joint evaluation of the consumer acceptance tests and the assessor's responses allows identifying the key drivers for a product acceptance [17].

Aim of the Study

The aim of this study was to evaluate the changes in intensity of sweet, bitter, sour and acerola flavor of acerola nectar prepared with sucrose and different sweeteners using time-intensity method, and investigate the parameters of the time-intensity curve affecting the consumer's preference.

Material and Methods

Materials

The acerola nectar samples were prepared by mixing one part of the pulp (Mais Fruta® - Jarinu, Brazil) to two parts of mineral water according to the manufacturer's instructions, in industrial blender (Sire® - Brusque, Brazil) for 1 minute, one day before the test. Samples were sweetened with sucrose and different sweeteners concentrations previously determined by magnitude estimation method [18] as follows: 1 g/L stevia extracts 40%, 80% and 95% rebaudioside A, and 0.99 g/L extract 60% rebaudioside A (Steviafarma® do Brasil - Maringá, Brazil); 0.16 g/L sucralose (Sweetmix® - Sorocaba, Brazil); 0.017 g/L neotame (Sweetmix® - Sorocaba, Brazil), and 80 g/L sucrose (União - São Paulo, Brazil).

Methodology

Pre-selection of assessors

The pre-selection was performed by triangular tests and sequential analysis [19]. Two samples of acerola nectar sweetened with 3.5% (w/v) and 5% (w/v) sucrose were used, presenting a significant difference of 1% probability determined by a paired comparison test performed with 30 panelists. The present study was approved by the ethics committee (CAAE: 01416412.0.0000.5404).

The parameters used in the sequential analysis were: p_0 = 0.45 (maximum unacceptable ability), p_1 = 0.70 (minimum acceptable ability), α = 0.05 (probability of accepting a candidate without sensory acuity) and β = 0.05 (probability of rejecting a candidate with sensory acuity). According to sequence analysis, 14 assessors, being 2 men and 12 women aged 20 to 34 years were pre-selected for the time-intensity analysis of the gustatory stimulus sweet, bitter, sour and acerola flavor.

Time intensity analysis

The gustatory stimulus sweet, bitter, sour and acerola flavor were analyzed for its intensity as a function of time (time-intensity analysis), separately for each sample [20].

Data collection was performed in computer, in a acclimatized room (22°C) using the software "Time-intensity Analysis of Flavors and Tastes - TIAFT" [21], developed at the Laboratory of Sensory Science and Consumer Research in the School of Food Engineering - UNICAMP.

Maximum reference levels for each stimulus were presented to the assessors. For sweet taste, acerola nectar sweetened with 10% sucrose was used as reference, while acerola nectar sweetened with 0.12% stevia extract 40% rebaudioside A was used as reference for bitter taste. The reference for acerola flavor was a nectar formulation containing 38% pulp and 62% water sweetened with 8% sucrose. Acerola nectar sweetened with 8% sucrose and added 0.2% citric acid was used as reference for sour taste.

Two sessions were carried out to familiarize the assessors with both the program and methodology. During the sessions, the in-mouth residence time and the total duration were established for each test. For all tests, the following parameters were used: initial waiting time: 10 seconds; in-mouth residence time: 10 seconds; intensity scale: 10. Time after ingestion was 90 seconds for sweet, bitter, acerola flavor and sour taste.

To select the team, a session with the seven samples of acerola nectar sweetened with sucrose and different sweeteners was conducted, in which the candidates were offered 10 mL sample in 50 mL disposable plastic cups, in three repetitions for each stimulus. Samples were presented at 6 ± 2°C on isothermal polystyrene trays, in a monadic order, and coded with three-digit random numbers. The assessors were instructed to drink water and wait for one minute between the evaluation of a sample and another. Assessors were selected according to their discriminatory power ($p < 0.50$), repeatability ($p > 0.05$) and agreement with the team [22]. Twelve assessors aged between 20 and 34 years were selected.

The assessors evaluated the samples in monadic form, with an interval of 3 days between sessions, in four replicates for each stimulus. The following parameters were evaluated: a) maximum intensity (Mi), b) time of maximum intensity (Tmi) c) total time duration (Ttot) and d) area under the time x intensity curve (Area).

Acceptance test

One hundred and twenty consumers, 79 women and 41 men aged between 17 and 60 years participated in the acceptance test, which assessed the overall impression of the samples. For this purpose, 30 mL of each sample were presented to consumers in a monadic order in balanced complete block [23], in 50 mL disposable plastic cups coded with three-digit random numbers at 6 ± 2ºC. A 9 cm unstructured hedonic scale with the extremes anchored as "dislike extremely" to "like extremely was used for the test [24].

Statistical analysis

The results of the parameters of each time-intensity curve were analyzed by ANOVA and Tukey's test at 5% significance level using the software SAS [25].

External preference mapping and partial least squares regression (PLSR) was applied to the results of the acceptance test associated with the time-intensity analysis using the software XLSTAT [26]. In the data matrix referring to these analyses, the attribute overall impression was the response variable and the parameters of time-intensity curve were considered predictive variables.

Results and Discussion

Time-intensity of sweet and bitter stimulus

The mean values of each parameter of the time-intensity curve for sweet and bitter taste are shown in table 1.

Stimulus	Parameter	Sucrose	Neotame	Sucralose	Stevia 40%	Stevia 60%	Stevia 80%	Stevia 95%
Sweet	I_{max}	6.28[c]	8.40[a]	6.49[bc]	7.21[abc]	7.43[abc]	7.74[ab]	7.72[ab]
	T_{Imax}	13.76[c]	8.40[d]	14.40[bc]	15.59[ab]	16.40[a]	15.83[ab]	7.72[d]
	T_{tot}	29.69[c]	52.72[a]	37.55[b]	48.83[a]	47.04[a]	51.02[a]	46.67[a]
	Area	100.59[d]	270.99[a]	155.08[c]	216.94[b]	207.18[b]	239.20[ab]	229.60[b]
Bitter	I_{max}	1.74[c]	4.21[b]	2.47[c]	5.93[a]	6.44[a]	5.90[a]	5.99[a]
	T_{Imax}	9.84[d]	14.53[abc]	11.83[cd]	14.61[ab]	14.67[ab]	15.95[a]	12.76[bc]
	T_{tot}	13.97[c]	31.67[b]	19.25[c]	39.71[ab]	42.44[a]	42.52[a]	39.62[ab]
	Area	26.11[c]	95.13[b]	40.11[c]	141.46[ab]	160.13[a]	160.29[a]	152.21[a]

Table 1: *Mean values of the parameters of the time-intensity curve for sweet and bitter stimulus.*
** Means followed by the same letter in the same row do not differ (p ≤ 0.05) by Tukey's test.*

Regarding the sweet stimulus, the sample sweetened with neotame showed the highest values for both maximum intensity (Mi) and total time (Ttot), not differing from the samples sweetened with different stevia extracts, while the lowest averages for these parameters were observed for the sample sweetened with sucrose. The highest value for the time of maximum intensity (Tmi) was observed for the sample sweetened with stevia extract 60% rebaudioside A, which did not differ from the samples containing stevia extract 40% and 80% rebaudioside A. In contrast, the samples containing neotame and stevia extract 95% rebaudioside A had the lowest value for this parameter. With respect to the area under the curve (Area), the sample sweetened with neotame had the highest value and did not differ from the sample sweetened with stevia extract 80% rebaudioside A. The smallest Area was found for the sample sweetened with sucrose (Table 1). According Goyal, Samsher and Goyal [27] the rebaudioside A is more sweeter and stable and less bitter than stevioside.

As can be seen in table 1, for the bitter stimulus, samples sweetened with different stevia extracts had higher mean values for maximum intensity (Mi). In addition, the highest value for the time of maximum intensity (Tmi) was observed for the sample sweetened with stevia extract 80% rebaudioside A, which did not differ significantly from the samples containing stevia 40% and 60% rebaudioside A and neotame. In contrast, the sample sweetened with sucrose showed the lowest value for this parameter and did not differ from the samples containing sucralose. Samples sweetened with stevia 60% and 80% rebaudioside A presented the highest values for total time (Ttot) for bitter stimulus, not differing from samples with stevia 40% and 95% rebaudioside A.

For the parameter Area under the curver (area), the highest values were found for the samples containing 60%, 80%, and 95% rebaudioside A, which did not differ significantly from the sample with stevia 40% rebaudioside A. The samples sweetened with sucralose and sucrose had the lowest mean values for maximum intensity (Mi), total time (Ttot) and area under the curve (Area).

The time-intensity curves for sweet and bitter stimulus of acerola nectar samples are presented in figure 1.

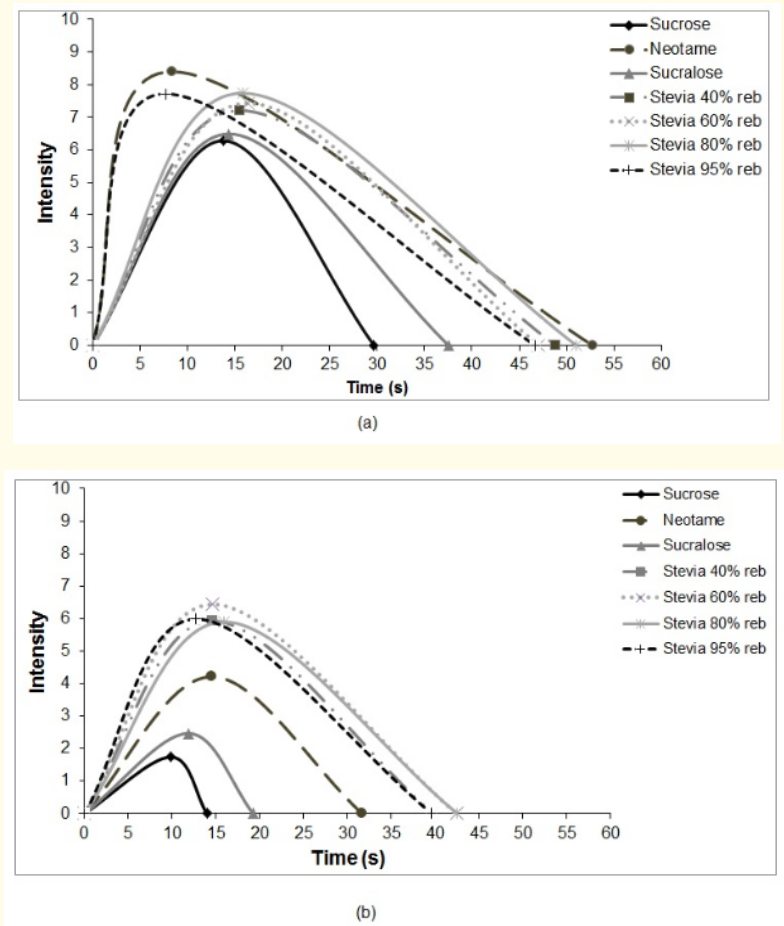

Figure 1: *Time-intensity curves for sweet (a) and bitter (b) stimulus of sweeteners and sucrose in acerola nectar.*

As can be seen in figure 1a, the sample sweetened with neotame was characterized by high maximum intensity (Imax), longer duration of sweet taste (Ttot) and together with the sample sweetened with stevia 95% rebaudioside A, it presented short maximum intensity (Timax). The sample prepared with sucrose stood out for the smallest total time (Ttot) and together with the sample sweetened with sucralose, it presented the lowest values for maximum intensity (Imax). It was found that the total time of sweet taste (Ttot) for the samples containing stevia 80% and 40% rebaudioside A were close to the sample sweetened with neotame. Acerola nectar samples presenting longer duration of sweet taste (Ttot) were prepared with Neotame and different stevia extracts, indicating that these samples have residual sweet taste.

Similar results were found by Cavallini and Bolini [28] in mango juice, in which the sucralose presented profile similar to that of sucrose for sweet stimulus.

According to figure 1b, samples containing stevia with 60% and 80% rebaudioside A stood out for longer duration of bitter taste (Ttot). The samples sweetened with stevia extracts were characterized by greater intensity of bitter taste (Imax). Acerola nectars with sucrose and sucralose highlighted by having lower intensity (Imax), while the sample sweetened with sucrose was characterized by shorter time of maximum intensity (Timax) for bitter taste. The time-intensity curve of the sample with sucralose was the one closest to the sucrose.

Cavallini and Bolini [28] and Brito and Bolini [29] reported that both samples of mango juice and guava nectar sweetened with stevia presented curves distant from the other samples, which were characterized by all parameters of the time-intensity curve for the bitter stimulus.

Time-intensity of sour and acerola flavor stimulus

Table 2 shows the mean results of each parameter of time-intensity curves for sour and acerola flavor stimulus.

Stimulus	Parameter	Sucrose	Neotame	Sucralose	Stevia 40%	Stevia 60%	Stevia 80%	Stevia 95%
Sour	I_{max}	5.21^{a}	5.24^{a}	5.47^{a}	5.48^{a}	5.34^{a}	5.40^{a}	5.21^{a}
	T_{Imax}	13.31^{a}	14.07^{a}	13.87^{a}	14.02^{a}	13.33^{a}	12.94^{a}	13.95^{a}
	T_{tot}	25.03^{ab}	24.59^{ab}	26.67^{a}	25.76^{ab}	24.04^{b}	26.34^{ab}	24.25^{b}
	Area	74.29^{ab}	66.55^{ab}	77.06^{ab}	77.82^{a}	69.65^{ab}	71.44^{ab}	60.09^{b}
Acerola flavor	I_{max}	6.93^{a}	6.58^{a}	6.63^{a}	6.34^{ab}	7.00^{a}	6.60^{a}	5.70^{b}
	T_{Imax}	13.98^{a}	13.48^{a}	14.22^{a}	13.13^{a}	13.42^{a}	13.38^{a}	13.60^{a}
	T_{tot}	30.68^{a}	25.47^{b}	28.63^{ab}	27.12^{ab}	24.65^{b}	26.66^{ab}	25.31^{b}
	Area	118.06^{a}	105.20^{a}	114.63^{a}	96.25^{ab}	98.70^{ab}	98.67$^{a.b}$	80.00^{b}

Table 2: *Mean values of the parameters of time-intensity curve for sour and acerola flavor stimulus.*
**: Means followed by the same letter in the same row, do not differ (p ≤ 0.05) by Tukey's test.*

As can be seen in table 2, the acerola nectar samples did not differ significantly with respect to parameters maximum intensity (Mi) and time of maximum intensity (Tmi) for the sourness. The sample prepared with sucralose showed the highest total time duration (Ttot), while the lowest average for this parameter was observed for the samples with stevia 80% and 95% rebaudioside A, and the remaining samples did not differ significantly from those with higher or lower values for total time duration (Ttot). Regarding the parameter Area under the curve (area) for the sour stimulus, the lowest value was found for the sample sweetened with stevia 95% rebaudioside A, and the highest value was observed for nectar containing stevia 60% rebaudioside A. The samples containing sucrose, sucralose, neotame and stevioside 40% and 60% rebaudioside A did not differ significantly from the samples presenting the highest and the smallest Area under the curve (Area).

The lowest mean value for the parameter maximum intensity (Mi) for the acerola flavor stimulus was observed for the sample sweetened with stevia 95% rebaudioside A, which did not differ from the sample containing stevia 60% rebaudioside A. There was no significant difference (p > 0.05) between the samples for the parameter time of the maximum intensity (Tmi). The highest total time duration (Ttot) for acerola flavor was found in the sample sweetened with sucrose, which was not significantly different from the samples prepared with sucralose, neotame and stevia 60% rebaudioside A. The nectar samples containing stevia extracts 40%, 80% and 95% rebaudioside A had the lowest mean values for this parameter. With respect to the area under the curve (Area) for acerola flavor stimulus, the highest averages were found for the samples sweetened with sucrose, sucralose and stevia extract 40% rebaudioside A, while the sample containing stevia extract 95% rebaudioside A presented the smallest area. Samples with neotame and stevia extracts 60% and 80% rebaudioside A did not differ significantly from samples presenting the highest and the smallest area under the curve (area) (Table 2).

The time-intensity curves for sour taste and acerola flavour of nectar acerola samples are presented in figure 2.

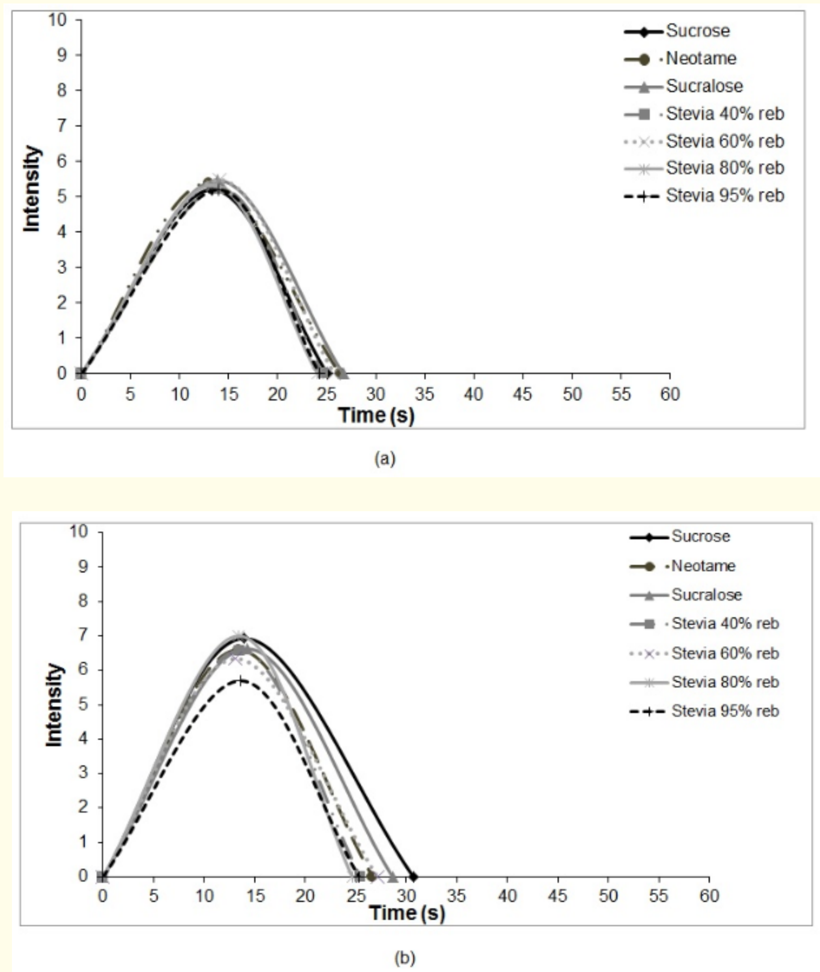

Figure 2: *Time-intensity curves for sour (a) and acerola flavor (b) stimuli of sweeteners and sucrose in acerola nectar.*

It can be observed in figure 2 that the time-intensity curves for the sour stimulus of the different samples were similar with regard to the maximum intensity and time to maximum intensity, and the samples sweetened with neotame and sucralose were highlighted for having the highest total time duration (Ttot) for the stimulus.

Sousa., *et al.* [30] studied the acid stimulus for traditional and light mango nectar, and found that light samples were characterized by the parameter time of the maximum intensity, while the parameters Area under the curve (area), total time (Ttot) and maximum intensity (Mi) characterized the traditional samples. Commercial samples of reduced calorie strawberry jam showed the highest values for maximum intensity (Mi) and the lowest values for time of maximum intensity (Tmi) for the acid stimulus [15].

According to figure 2, the time of maximum intensity (Timax) was similar for the different acerola nectar samples. Sample sweetened with stevia 95% rebaudioside A highlighted by the lowest values for maximum intensity (Imax), while samples containing sucrose and stevia with 80% rebaudioside A had the highest values for this parameter. The largest total time (Ttot) of acerola flavor was higher for the sample sweetened with sucrose and close to the nectar sweetened with sucralose. In addition, it was observed that the samples sweetened with stevia extracts containing 40%, 80% and 95% rebaudioside A showed total duration (Ttot) of acerola flavor similar and lower than the other samples.

For guava flavor, the parameters Area under the curve (area) and maximum intensity (Mi) were higher for the sample of guava nectar sweetened with sucrose, being very similar to the nectar containing sucrose [29].

Parameters affecting the acceptability

Figure 3 shows the external preference mapping of the overall impression and the averages of the time-intensity curve for sweet taste, bitter taste, sour taste and acerola taste.

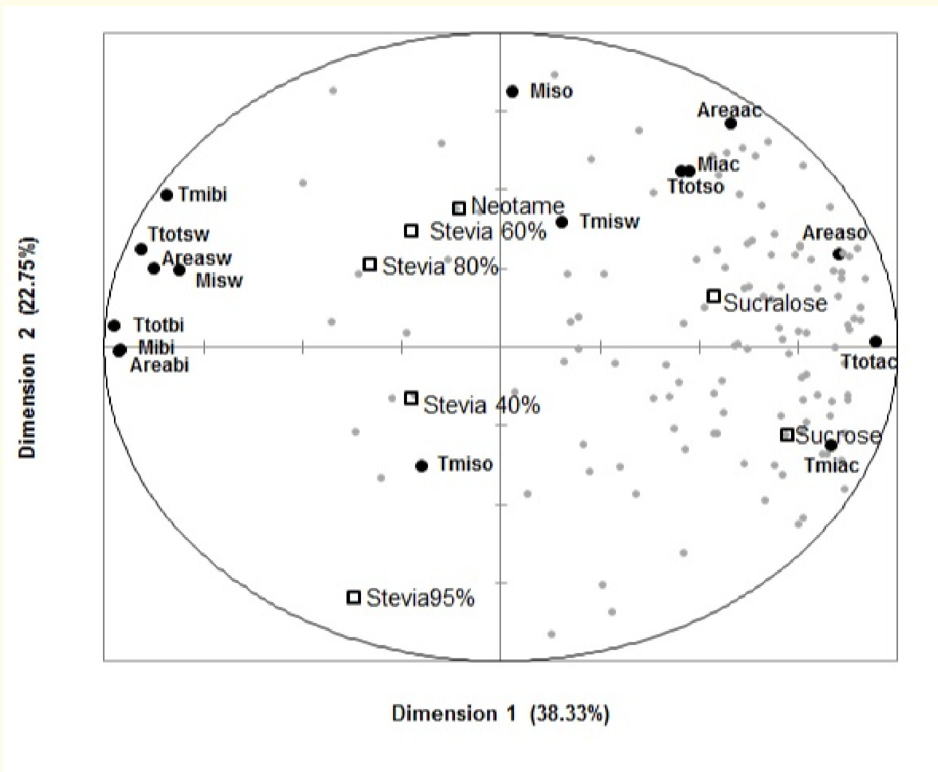

Figure 3: *External preference mapping of the overall impression, and the averages of the time-intensity curve for sweet taste, bitter taste, sour taste and acerola taste.*

Legend: Ttotsw: Total Time of Sweet Stimulus; Tmisw: Time of Maximum Intensity of Sweet Taste; Misw: Maximum Intensity of Sweet Taste; Areasw: Area Under the Time-Intensity Curve of Sweet Taste; Ttotbi: Total Time of Bitter Stimulus; Tmibi: Time of Maximum Intensity of the Bitter Taste; Mibi: Maximum Intensity of Bitter Taste; Areabi: Area Under the Time-Intensity Curve of Bitter Taste; Ttotac: Total Time of Acerola Flavor Stimulus; Tmiac: Time of Maximum Intensity of Acerola Flavor; Miac: Maximum Intensity of Acerola Flavor; Areaac: Area Under the Time-Intensity Curve of Acerola Flavor; Ttotso: Total Time of Sour Taste Stimulus; Tmiso: Time of Maximum Intensity of Sour Taste; Miso: Maximum Intensity of Sour Taste; Areaso: Area Under the Time-Intensity Curve of Sour Taste.

Sample sweetened with sucrose showed a higher correlation with the parameter time of the maximum intensity of acerola flavor (Timsa), while the sample sweetened with sucralose was correlated with the parameters total time of acerola flavor (Ttotsa), and area under the time-intensity curve of acerola flavor (Areasa), suggesting that these parameters of the time-intensity curve can be positively influenced the acceptance of these samples. The parameter time of maximum intensity of sweet taste (Timdo) showed the highest correlation with the samples sweetened with neotame and stevia extract with 60% rebaudioside A, while samples sweetened with stevia 40% and 95% rebaudioside were correlated with the parameter time of the maximum intensity of sour taste (Timac). The sample containing stevia with 80% rebaudioside A was correlated with the maximum intensity of sweet taste (Imaxdo) and area under the curve of sweet taste (Areado), indicating that these parameters may have influenced the low acceptance of these samples.

The partial least squares regression, with a confidence interval of 95% is shown in figure 4.

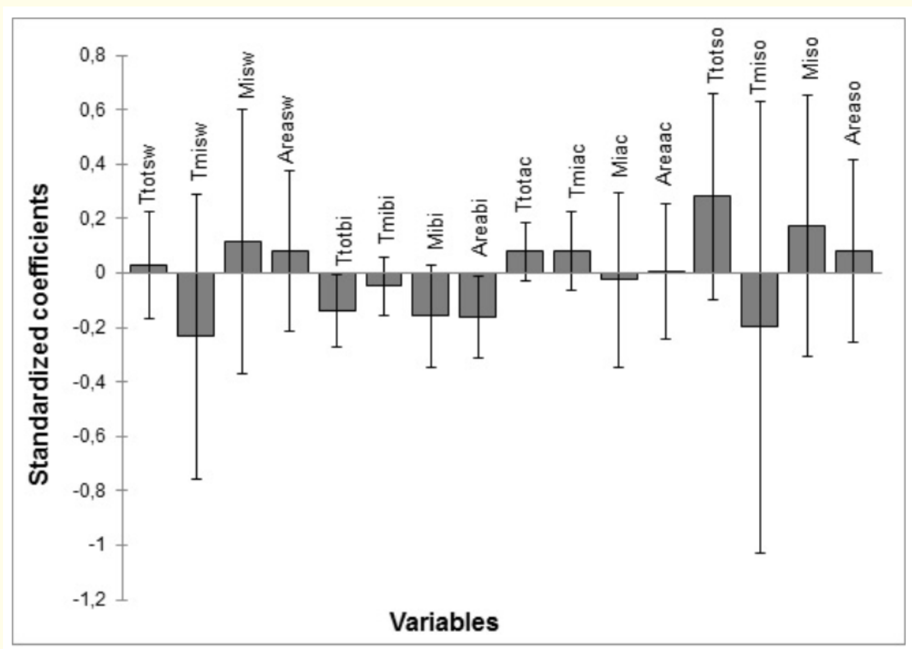

***Figure 4:** Standardized coefficients by partial least squares regression between the parameters of time-intensity curve and overall impression.*

Legend: Ttotsw: Total Time of Sweet Stimulus; Tmisw: Time of Maximum Intensity of Sweet Taste; Misw: Maximum Intensity of Sweet Taste; Areasw: Area Under the Time-Intensity Curve of Sweet Taste; Ttotbi: Total Time of Bitter Stimulus; Tmibi: Time of Maximum Intensity of the Bitter Taste; Mibi: Maximum Intensity of Bitter Taste; Areabi: Area Under the Time-Intensity Curve of Bitter Taste; Ttotac: Total Time of Acerola Flavor Stimulus; Tmiac: Time of Maximum Intensity of Acerola Flavor; Miac: Maximum Intensity of Acerola Flavor; Areaac: Area Under the Time-Intensity Curve of Acerola Flavor; Ttotso: Total Time of Sour Taste Stimulus; Tmiso: Time of Maximum Intensity of Sour Taste; Miso: Maximum Intensity of Sour Taste; Areaso: Area Under the Time-Intensity Curve of Sour Taste.

It can be observed in figure 4 that the parameters of the time-intensity curve, total time of bitter stimulus (Ttotbi) and area under the time-intensity curve of bitter stimulus (Areabi) had a negative effect on the overall impression scored by acceptance test. According to table 1, samples sweetened with stevia 60% and 80% rebaudioside A presented the highest values for both total time (Ttotbi) and area under the time-intensity curve of bitter stimulus (Areabi), along with the sample sweetened with stevia 95% rebaudioside A.

The bitter taste and bitter aftertaste of *Stevia rebaudiana* are characteristics that affect the sensory acceptance of products sweetened with this sweetener [29,31]. In addition, it is known that stevia masks the flavor in fruit juices and fruit nectars [28,29]. However, according to Silvia [32] some consumers prefer to consume stevia extract as a natural sweetener and lose in sensory quality, rather than consume synthetic sweeteners. Knowledge of the parameters of the time-intensity curve influencing the acceptance of a product proves to be extremely valid to develop new products and improve existing products in food industry.

Conclusion

The results for the time-intensity analysis for the sweet and bitter stimulus of the acerola nectar showed that the sample sweetened with sucralose presented temporal profile closer to the nectar sweetened with sucrose. For both sour taste and acerola flavor, all nectar samples showed similar temporal profiles, with the exception of the nectar sample sweetened with stevia 95% rebaudioside A.

The parameters of the time-intensity curve, total time of bitter stimulus (Ttotbi) and area under the time-intensity curve of the bitter stimulus (Areabi) negatively influenced the acceptance of acerola nectar.

Acknowledgements

The authors are grateful for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarship to first author.

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