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Evaluation of the potential use of rebaudioside-A as sweetener for diet jam

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Abstract

Sweeteners based on stevia extract contain a series of diterpene glycosides derivatives from steviol, standing out the rebaudioside-A. There is no tabletop sweeteners in the market formulated purely with rebaudioside-A yet, so its use in foods depends on the development of new products followed by physicochemical and sensory evaluations. This work presents the formulation of a diet strawberry jam dyed with cranberry juice and sweetened with rebaudioside-A purified from stevia plants of the lineage UEM-320 developed in the Centro de Estudos de Produtos Naturais da Universidade Estadual de Maringá. Evaluations of physicochemical properties, microbiological and sensory characteristics were carried out for the product in comparison with a control sweetened with equal amount of sucralose. The results showed that the physicochemical characteristics of the sample and the control are not significantly different and the supplementation with cranberry juice increased both color and total phenolic content in both samples. The sensory acceptability indicated a significant preference for the formulation sweetened with 100% of rebaudioside-A, only in the items flavor and purchase intent. We concluded that rebaudioside-A has a better sensory performance than sucralose, even this last one being 1.33 fold sweeter than rebaudioside-A.

Keywords: rebaudioside-A; steviol glucosides; sweetener.

1 Introduction

Stevia rebaudiana (Bert.) Bertoni is a plant of the family Asteraceae. This plant has in its leaves, stems and flowers a series of diterpene glycosides, a group of sweeteners with a common core called steviol. These sweeteners differ primarily in the number and conformation of glucose moieties attached to the steviol core (HORN, 2012), being the stevioside and rebaudioside-A (Reb-A) the most important regarding their sweetening properties which are 300- and 450-fold sweeter than sucrose respectively, lying on the threshold of the perception of the sweet flavor (WELLS, 1989; BRANDLE; STARRATT; GIJZEN, 1998).

Among the sweeteners in stevia, the Rebaudioside-A (CID: 56840979) is the most stable, sweeter (450-fold the sweetness of sucrose and 1.5-fold the sweetness of stevioside), it has good water solubility and also has a sensory profile closer to the sucrose, bringing out several competitive advantages compared to stevioside (GOTO; CLEMENTE, 1998).

The *Núcleo de Estudos em Produtos Naturais* (NEPRON) from the State University of Maringá (UEM) has developed a series of clones and varieties of stevia with high content of rebaudioside-A, highlighting the clonal line of Stevia UEM-320 and the variety M1Alvarez, being this last one capable to be multiplied by its seeds (ALVAREZ; COUTO, 1984; COSTA, 2004; PAULA, 2006; LOPES, 2007). These plants have been our main source of Reb-A.

On the other hand, Sucralose (CID: 71485) is a sweetener that, although artificial, has excellent sensory profile and shares a considerable part of the worldwide commerce for high intensity sweeteners. Sucralose is a powerful and high quality sweetener stable at a wide range of pH. It is non-caloric and is obtained from the chlorination of sucrose resulting in a stable product with excellent physical and chemical characteristics that allows its application in various foods and beverages (SHIBAO et al., 2009).

Since the utilization of rebaudioside-A as a food sweetener becomes an alternative to the use of sucralose, it is necessary that the technology for using this substance in foods be continuously improved by the development of new products and its subsequent evaluation by its sensory and physicochemical properties.

The choice for jams in this work was justified by the fact they are considered the second commercially important product for the industry of canned fruit in Brazil. In other countries such as the European countries, these products stand out both in consumption and in their quality. The processing of fruit into manufactured products allows the absorption of much of the harvest, a fact that encourages the consumption throughout the year and also contributes to reduce the waste of food (CAETANO; VIEITES, 2010).

The American cranberry (*Vaccinium macrocarpon* Ait. Ericaceae) can be considered a functional food due to the powerful health benefits of many of its compounds. The cranberries have in their composition fibers, glucose, fructose,

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vitamin-C, organic acids, flavonoids, and a high level of anthocyanins and proanthocyanidins. In spite of the fact that the juice and/or fresh fruit have been consumed long ago to prevent infections of the urinary tract and also as natural antioxidants acting against some types of cancer (McHARG; RODGERS; CHARLTON, 2003; VINSON et al., 2001; JEPSON; WILLIAMS; CRAIG, 2012; DUTHIE et al., 2006; SEERAM et al., 2006) the exploitation of its nutraceutical properties was not the objective of this work. The main objective in the use of cranberry juice in the formulations was to enhance the natural red color of the product as well to give it some flavor.

Therefore, the main objective of this work was to compare the acceptability of two formulations of diet strawberry jam colored with concentrated cranberry juice, one formulation sweetened with rebaudioside-A extracted from the stevia line UEM-320 and other with sucralose. The final formulations were subjected to physicochemical, microbiological, total phenolics and sensory evaluations aiming to contribute to the development of a direct application for the Reb-A technology on foods and launch alternatives for innovative products as a consequence.

2 Materials and methods

2.1 Jam production

Jams were prepared in the fruit processing unit at the Department of Technology from the State University of Maringá (DTC/UEM) – campus of Umuarama between August and September, 2011. Fresh fruit were purchased at a local market and sanitized following the ANVISA recommendations for good manufacturing practices. The formulations were prepared using 300 g strawberries, 5 g low methoxy pectin, 5 mL cranberry juice at 40° Brix (Agricola CranChile Ltda., Lanco, Chile) and 100 mL water. All ingredients were homogenized in a blender for five minutes and then subjected to cooking. After that, ascorbic acid (300 mg, Merk), citric acid (100 mg, Reagen) and sodium chloride (50 mg, Reagen) were added. For the final formulation F1, 500 mg Reb-A were added, and for the final formulation F2, 500 mg Sucralose (Linea) were added. Finally, sterilized glass bottles were filled with the jams, closed with metal lids, pasteurized into boiling water at 100 °C for 15 minutes, inverted and allowed to cool at room temperature. The bottles were stored for 30 days simulating shelf life before further analysis. A reference sample without neither cranberry nor antioxidant additives was produced to be used as control in some evaluations.

2.2 Physicochemical evaluations

These analyses were performed at the Laboratory of Physical Chemistry from DTC/UEM, only for the final formulations. The values presented here are the simple average and standard deviation from three assays. Variance analysis between results from the two formulations was made by ANOVA.

Jams were analyzed for proximate composition (moisture, total carbohydrates, proteins, ether extract, crude fibers and total ash) according to the methodologies of *Instituto Adolfo Lutz* (2008). The content of total carbohydrates was calculated by the difference from the other composition fractions. The control samples, free of citric and ascorbic acids, were used to evaluate the Total phenolic content according to methodologies of AOAC and the modifications suggested by Morelli and Prado (2011). All spectrophotometric analyses were made by a Cary-50Scan (Varian Inc., Palo Alto, USA) spectrophotometer.

Absolute color

Differences in color were determined by using the CIE $L^*a^*b^*$ color system by a Minolta Chroma Meter CR-400 (Minolta Co., Japan) only for the final formulations. CIE $L^*a^*b^*$ model uses a system of rectangular coordinates that defines the color in terms of lightness (L^*), red versus green (a^*) and yellow versus blue (b^*). The L^* represents the difference between light (L^*=100) and dark (L^*=0). The component a^* represents the difference between green ($-a^*$) and red ($+a^*$) and grey corresponds to zero. The component b^* represents the difference between blue ($-b^*$) and yellow ($+b^*$) and zero corresponds to gray (SAHIN; SUMNU, 2006).

2.3 Microbiological analysis

All microbiological analyses were performed at the Laboratory of Microbiology from DTC/UEM campus Umuarama-PR, according to the methodology proposed by Silva, Junqueira and Silveira (2001), and the recommendations of RDC nº 12/2001 from ANVISA (AGÊNCIA..., 2001) for fruit jam, evaluating molds and yeasts per gram of sample.

2.4 Ethical aspects

Tests of sensory properties involving panelists were conducted after approval of the project by the ethics committee of UEM, protocol number 082/2009.

2.5 Sensory evaluations

The sensory evaluations were performed at the Sensory Laboratories 1 and 2 from DTC/UEM. The tests were performed within individual booths in an air-conditioned environment under white light and the samples were offered in a randomized monadic sequential presentation.

Aiming to find the optimal concentration of Reb-A to be added in the formulations, a series of preliminary tests with formulations containing Reb-A 100%, 75% Reb-A + 25% Sucralose and 50% Reb-A + 50% Sucralose were made. These formulations were tested by 54 untrained panelists. To avoid biased results, samples were presented in different positions and balanced, with random repetitions, totaling 9 trials for each combination.

After finding the final formulation with Reb-A (F1), an equal amount of sucralose was used in the reference formulation (F2), without considering the sweetener capability. Then, sensory comparative tests were performed. To avoid biased results, samples in this new test were again presented to panelists in different balanced positions with randomized repetitions, as previous described, with a total of 50 tests for each combination using 100 untrained panelists different from individuals previously enrolled in the affectivity test.

To verify the acceptability and preference for the jams, a 9 point hedonic scale (1 = dislike extremely) (9 = like extremely) was used. The attributes evaluated were their overall appearance, aroma, flavor and texture. In addition, the purchase intention of the product according to scale (5 = definitely buy; 1 = definitely not buy) was verified. The sensory panel consisted of 100 untrained people of both genders aging from 15 to 55 years old. Panelists were previously instructed about how to fill out the evaluation form and, in order to carry out the analysis they received samples (approximately 20 g) in white dischargeable cups coded with random numbers plus the evaluation form. The results were subjected to analysis of variance (ANOVA) at 0.05 level of significance, processed using the software Origin®.

3 Results and discussion

The incorporation of Reb-A in the heated mass (approximately 70 °C) did not affect its structural characteristics. This assurance comes from experiments where equivalent amounts of Reb-A dissolved in water were subjected to temperatures of 25 °C (control), 60°, 80° and 100° C for 15 minutes and after this period, analyzed by HPLC. No change was observed in the chromatographic profiles at different temperatures (data not shown).

A few papers were found in the literature regarding proximate characterizations of low calories jams. This fact adds certain importance to this work and also justifies the comparison of our results with other works with products of similar composition.

Table 1 presents the results from the physicochemical evaluations of the diet jams. As expected for products made with the same basis, the composition of jams F1 and F2 had no significant differences ($p>0.05$) for all aspects evaluated.

Despite the lack of significant difference ($p>0.05$) in the average pH between the two formulations, the slightly lower mean pH value found in F1 may be because Reb-A presents a greater number of H-bond donor groups (14 H-bond donors) than Sucralose (5 H-bond donors). The literature recommends that the pH of the jams should be lower than 3.40; however, at values below 3.00 there is a tendency to syneresis (JACKIX, 1988). Although the pHs of the jams produced in this work have been slightly above recommendations, no microbiological contamination was observed, indicating that those slightly high pH values did not interfere with the objective of the acidulant agent in the sample. Nevertheless the pH values found herein are very similar to 3.69 found in light strawberry jam (ZAMBIAZI; CHIM; BRUSCATTO, 2006), and 3.56 from light pineapple jam (GRANADA et al., 2005).

The bibliography regarding the composition of jams produced with low methoxy pectin describes that such products may turn into gel at concentrations of soluble solids ranging from 10-70%, pH from 2.00 to 6.00 (TORREZAN, 1998), and soluble solids concentration up to 25° Brix (CAMPOS; CÂNDIDO, 1995). Regarding these characteristics, the formulations prepared in this study are in accordance with the recommendations. A study by Polesi et al. (2011), made with diet mango jam showed values corresponding to 89.40% moisture, ash 0.44%, soluble solids of 11° Brix and carbohydrate content of 6.39%. Taking those values as references, the formulations prepared in this study are similar to that described in the literature.

Color parameters from the strawberry-cranberry jams were significantly different from those found in the reference sample with strawberry ($p<0.05$). The lower value of L^* found in F1 and F2 formulations means a darker product, while the higher value of a^* and b^* means an increase in the red color (Table 2), which made the product more visually appealing for the panelist.

Regarding the microbiological evaluations, the jams were tested only for molds and yeasts (UFC g^{-1}). The results show no contaminations after a period of 30 days quarantine simulating shelf life. It means that the manufacturing techniques adopted here were appropriate for both formulations and bring security to the panelists who volunteered for the sensory tests. Due to the low thermal resistance of molds and yeasts, these agents are rarely associated with the deterioration of products that underwent thermal treatment (GRANADA et al., 2005).

3.1 Total phenolic quantification

The cranberry is a fruit known for having several nutritional advantages (NETO, 2007). Its recognized antioxidant activity is one of the most important (VINSON et al., 2001; BASU et al., 2011). The addition of the concentrated cranberry juice into the formulations aimed to act as a natural dye. However a

Table 1. Proximate and physicochemical characterization of the jams.

Analysis (g 100^{-1} g sample)	Jam F1 (Reb-A) Average ± sd	Jam F2 (Sucralose) Average ± sd
Moisture (%)	86.94 ± 1.69^a	84.86 ± 1.15^a
Total carbohydrate (%)	5.03 ± 0.74^a	6.08 ± 0.75^a
Protein (%)	1.31 ± 0.08^a	1.35 ± 0.02^a
Ether extract (%)	0.58 ± 0.01^a	0.53 ± 0.14^a
Crude fiber (%)	5.58 ± 0.78^a	6.64 ± 0.75^a
Total ash (%)	0.54 ± 0.08^a	0.54 ± 0.01^a
Citric acid (%)	1.60 ± 0.08^a	1.48 ± 0.09^a
Soluble solids (°Brix)	12.10 ± 0.84^a	13.30 ± 0.85^a
pH	3.66 ± 0.04^a	3.73 ± 0.03^a

Values with different letters in the row are significantly different at 5% level by the ANOVA test.

Table 2. Color parameters from the diet jams as $L^*a^*b^*$ system.

CIE parameter	Jam F1 (Reb-A) Average ± sd	Jam F2 (Sucralose) Average ± sd	Strawberry jam (Control) Average ± sd
L^*	33.94 ± 2.21^a	30.96 ± 2.15^a	47.11 ± 3.40^b
a^*	26.10 ± 0.55^a	26.69 ± 0.43^a	21.56 ± 1.94^b
b^*	14.56 ± 0.05^a	14.50 ± 0.05^a	13.48 ± 2.22^a

Values with different letters in the row are significantly different at 5% level by the ANOVA test.

predictable consequence of this addition was the increase in antioxidant agents like the total phenolic content (SAWA et al., 1999). The total phenolic levels found in the formulations of this work are listed in Table 3. Our results showed that there was an average increase of 46% in the levels of total phenolic in F1 and F2 jams compared to control, which is clearly attributed to the addition of cranberry juice. The observed variations between F1 and F2 were not significant (p>0.05) considering the size of the standard deviation.

Among the group of vegetables rich in phenolic compounds, red fruit (or berries) are one of the most important sources of dieting (PINTO; LAJOLO; GENOVESE, 2007; SÁNCHEZ-PATÁN et al., 2012). In Brazil, strawberries are the most important red fruit produced and consumed, while in North America the fruit with this role is the cranberry (STRIK, 2006). The cranberry is a well-known source of concentrated dietary flavonoids, including anthocyanins, flavonol glycosides and proanthocyanidins (condensed tannins), and various phenolic acids. Its main anthocyanin pigments are cyanidin and peonidin galactoside (TARASCOU et al., 2011). The most abundant flavonol glycosides in its extract are quercetin and mircetin, while the most abundant phenolic acids are ferulic, *p*-coumaric, caffeic and sinapic acids (TUMBAS et al., 2006). Most of the phenolics found in the cranberry are soluble as the free form (91.30 to 96.20%) and this is one of the few fruits containing a high proportion of phenolics (McKAY; BLUMBERG, 2007).

3.2 Sensory evaluation

Formulations

The main objective of this study was to evaluate the acceptability of diet products sweetened with Reb-A through sensory analysis. The first step was to find the ideal concentration and proportion of the sweetener to be used by affectivity tests. The tests indicated a slight preference for the formulation with 100% Reb-A in all aspects analyzed. Although the preference for this formulation was not significant for the most part, we chose to use it in the comparison tests with sucralose, once there was a clear preference for this formulation as suggested by the mean values from flavor and purchase intent aspects (Table 4).

Tests of acceptability by structured scale

After finding the optimum concentration of Reb-A to be used in the definitive formulations, we proceeded to evaluate the sweetener preference. The results of this evaluation are summarized in Table 5.

The analysis of the results in Tables 4 and 5, at first glance, may lead to the wrong impression that the purchase intention is low compared to the dimensions of the other aspects evaluated. However, according to the scales utilized, the purchase intention aspect ranges from 1 to 5 while the other aspects range from 1 to 9.

The comparison of the sensory performance of the two formulations clearly shows that the sample containing Reb-A was preferred in all aspects analyzed, however, the variables which show a significant difference at 5% probability by the ANOVA test (p<0.05) were again the flavor and purchase intent.

Table 3. Total phenolic quantification.

Product	Total Phenolic (mg EAG 100^{-1} g)
Jam F1 (Reb-A)	2.88 ± 0.28[a]
Jam F2 (Sucralose)	3.10 ± 0.30[a]
Strawberry jam (control)	2.04 ± 0.16[b]
Cranberry juice	16.14 ± 0.19

Values with different letters in the column are significantly different at 5% level by the ANOVA test.

Table 4. Mean values of sensory attributes for diet strawberry-cranberry jams.

Aspect evaluated	Formulation		
	Reb-A 100% Sucralose 0%	Reb-A 75% Sucralose 25%	Reb-A 50% Sucralose 50%
General appearance	7.0 ± 1.30[a]	6.9 ± 1.36[a]	7.0 ± 1.47[a]
Aroma	7.3 ± 1.20[a]	7.2 ± 1.38[a]	7.0 ± 1.48[a]
Flavor	6.6 ± 1.80[a]	6.5 ± 1.83[a]	5.9 ± 1.99[a]
Texture	6.8 ± 1.59[a]	6.9 ± 1.49[a]	6.5 ± 1.83[a]
Purchase intent*	3.6 ± 0.97[a]	3.5 ± 0.95[a]	3.3 ± 1.00[a]

Values with different letters in the row are significantly different at 5% level by ANOVA test. *Scale ranging from 1 to 5.

Table 5. Mean values of sensory attributes for diet strawberry-cranberry jams.

Aspect evaluated	Formulation	
	F1 (Reb-A)	F2 (Sucralose)
General appearance	7.6 ± 1.27[a]	7.4 ± 1.39[a]
Aroma	7.4 ± 1.50[a]	7.3 ± 1.47[a]
Flavor	6.7 ± 1.79[a]	5.5 ± 1.98[b]
Texture	7.1 ± 1.54[a]	6.9 ± 1.62[a]
Purchase intent*	3.5 ± 1.12[a]	2.9 ± 1.06[b]

Values with different letters in the row are significantly different at 5% level by the ANOVA test. *Scale ranging from 1 to 5.

Since both formulations were prepared from the same basis, differences in visual and smell aspects were not expected, since both sweetener Sucralose and Reb-A are odorless, have good solubility at the concentrations used and, therefore, did not lead to changes in odor nor induce the formation of granules, two characteristics that would influence the preference of the panelists. Therefore, these results clearly demonstrate the preference for the formulation with Reb-A.

This result is very surprising and becomes more interesting because Sucralose is about 1.33 fold sweeter than Reb-A (CARDOSO; CARDELLO, 2003; PSZCZOLA, 1999). Nevertheless, to find the best formulations presented in this study, a gravimetric criterion was adopted, i.e., we used equal quantities in weight from both sweeteners without taking into account the degree of sweetness. This criterion should give a clear advantage to the formulation containing Sucralose regarding the sweet flavor. Even so, the preference of the panelists regarding this criterion was still for the formulation with Reb-A.

There was a free space at the back of the evaluation form in which the panelists could leave comments about the products or the experiment. From these comments it was verified that for some people, the sour flavor of the jams determined the preference, while for others it was a cause of loss of choice and dissatisfaction. However, this is a very personal characteristic of the panelist, which justifies the scores ranging from 1 to 9 in individual assessments for the flavor of these products. However, the overall average of the attribute flavor for the jam with Reb-A was between six (like slightly) and seven (like moderately), while for the jam with Sucralose, the average was between five (nor liked / nor disliked) and six (like slightly).

4 Conclusions

The physicochemical and microbiological analysis of diet strawberry-cranberry jams lead to the conclusion that these products stand for a good option for the utilization of strawberry and also an alternative for the supply of low calorie products. They can also be leveraged as a good source of antioxidants for a specific audience, such as diabetic or obese people. None of the products show any microbial contamination after 30 days quarantine, stating that they were prepared under good sanitary conditions.

The addition of concentrated cranberry juice to the formulations was able to produce an intense red color and may be used as a natural alternative to synthetic dyes.

The formulations F1 (sweetened with Reb-A only) and F2 (sweetened with Sucralose only) presented similar physical and chemical characteristics and a good acceptance by the consumers. However, under the conditions used in this work, Reb-A had a significantly better sensory performance concerning flavor and purchase intent than Sucralose, which is the world leader in the market of high-intensity sweeteners.

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