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Substituting sucralose with rebaudioside A in soy foods: equivalent sweetness, physicochemical analysis, microbiological assessment and acceptance test

Cândyce Camile Fortuna NALESSO-LEÃO[1], Paula Gimenez MILANI[2], Maysa FORMIGONI[1], Maria Rosa Trentin ZORZENON[1]*, Antonio Sérgio DACOME[2], Antonio Roberto Giriboni MONTEIRO[3], Silvio Claudio da COSTA[2]

Abstract

Stevia rebaudiana (Bert.) Bertoni contains steviol glycosides with emphasis to rebaudioside A, which presents a stronger sweet flavor, more stability, good solubility and lower bitter residual taste, which benefits its application in foods and drinks. The objective of this study was to determine the equivalent amount of rebaudioside A necessary to promote the same degree of sweetness of sucralose in the grape- and peach-flavored soy beverages and evaluate their physicochemical characteristics and consumer acceptance. At the Natural Products Center of the State University of Maringa, the rebaudioside A was purified from the M1 Alvarez variety of Stevia plants. Through sensory analysis established that the ideal quantity of rebaudioside A to substitute sucralose in foods with peach soy and grape soy would be 3 times the quantity of sucralose. The results revealed no signifcant differences for the physicochemical and sensorial characteristics ($p < 0.05$) between the two formulations. The results also indicate that the rebaudioside A with high purity rate (97%) obtained from selected stevia varieties can substitute sucralose in peach soy foods and grape soy foods.

Keywords: sucralose; foods with peach soy; foods with grape soy; *Stevia rebaudiana*; sweeteners.

Practical Application: Artificial sweetener substitution by natural sweetener without prejudice to flavor.

1 Introduction

Stevia rebaudiana (Bert.) Bertoni is a plant of the family Asteraceae, South America native that has been exploited on large commercial scale since 1970, when stevia sweeteners were introduced in the Japanese market; in 1986, it had already accounted for over 40% of the intense sweeteners market of the country (Dacome et al., 2005). Consumers' demand for natural food, especially low-calory, has made the plant increasingly popular over the past years.

The leaves, stems and inflorescences of stevia present a series of diterpene glycosides, steviol glycosides with emphasis to stevioside and rebaudioside A (RebA) (Lemus-Mondaca et al., 2012). These glycosides have sweeteners properties 300 to 450 times sweeter than sucrose on the threshold of sweet taste, respectively (Pawar et al., 2013).

Among the steviol glycosides used in food products, the RebA is sweeter, more stable and presenting a sensory profile more similar to the sucrose with good water solubility enabling its use with a great variety of formulations (Williams & Burdock, 2009; Reis et al., 2011). In addition to these important properties, these non-caloric natural sweeteners have antioxidant and insulinotropic properties and may add functionality to foods or beverages (Milani et al., 2016). The Natural Products Studies Center (NEPRON) of the State University of Maringa through partnerships with the private sector and with the support of funding agencies set up a pilot unit with capacity to process 120 kg of stevia leaves per day and has developed many varieties of stevia with high RebA rate, highlighting the variety M1 Alvarez.

To consolidate the stevia market depends mainly on the use of RebA, the development of new formulations obtaining products with equal or better performance than other sweeteners, natural or synthetic, such as sucralose, synthetic sweetener obtained through chlorinating sucrose, which sensory properties and chemical allowed its wide-scale application in food and beverages. Although sucralose was initially considered safe to use, Oliveira et al. (2015) evaluated the stability of this sweetener and showed that the structure of sucralose can hydrolyze into toxic compounds when exposed to severe temperature conditions, forming chloropropanols and other related chlorinated compounds, which may generate possible harmful biological effects exhibited by this sweetener.

Soy is one of the most important agricultural products in Brazil; however, Brazilian diets do not apply soy expressively. The search for more nutritious foods tends to increase as more consumers feel motivated to seek products without cholesterol, less fat and low calorie. Studies point to the great health benefits that soy foods can provide, with emphasis to the water-soluble

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[1] *Programa de Pós-graduação em Ciência de Alimentos, Universidade Estadual de Maringá – UEM, Maringá, PR, Brasil*
[2] *Departamento de Bioquímica, Universidade Estadual de Maringá – UEM, Maringá, PR, Brasil*
[3] *Departamento de Engenharia de Alimentos, Universidade Estadual de Maringá – UEM, Maringá, PR, Brasil*
*Corresponding author: mariarosazorzenon@hotmail.com

extracts of soybean (EHS), with possible commercialization as sterile and pasteurized, with or without flavor addition (Barnes, 1998; Friedman & Brandon, 2001). According to Brasil (2000), the main source of protein in soy foods is actually the soy, so the product should contain soy extract, and optional pulps, juices and fruit concentrates.

In this context, the objective of this study was to compare the acceptability of drink with soy, named by Brazilian law, soy foods, added with concentrated grape juice and concentrated peach juice, sweetened with RebA and evaluate its sweetness potential when compared to the sucralose (sweetener widely used in this kind of drink). Having been established, these end formulations were submitted to microbiological, physicochemical analysis, and sensory assessment.

2 Materials and methods

2.1 Materials

RebA was obtained by extraction, isolation and purification of the *Stevia rebaudiana* leaves of the clonal variety M1 Alvarez grown at UEM. The ingredients of the formulations of soy beverages listed below were supplied by the company COCAMAR (Maringa, Parana, Brazil). Reagents for physicochemical and microbiological analyses are the *Sigma* brand. The ingredients used in the formulations (patent COCAMAR) are listed below:

- Food with grape soy - water, soy extract, concentrated grape juice, pectin, citric acid, ascorbic acid, dye cochineal carmine, aroma identic to the natural grape and sucralose (13 mg/100 mL) (COCAMAR patented product);
- Foods with peach soy - water, soy extract, clarified and concentrated peach juice pectin, citric acid, ascorbic acid, identical to natural peach aroma, dye cochineal carmine and turmeric and annatto dye and sucralose (15 mg/100 mL) (COCAMAR patented product).

All ingredients were homogenized in a blender for ten minutes and stored less than 5 °C for to conduct the analyses.

2.2 Preparation of the formulations and equivalent sweetness

The soybean food base grape and peach flavor was produced according to a method developed and patented by the company COCAMAR. The formulations regarding the applied sweetener: sucralose and Reb A. RebA was obtained through extraction, isolation and purification of *Stevia rebaudiana* leaves of the clonal variety M1 Alvarez grown at NEPRON, using the method developed by laboratory researchers (Costa et al., 2005). Sucralose was provided by COCAMAR and the quantity of added sucralose had been established in the description of the ingredients list in the commercial product package: 13 mg/100 mL for the food with grape soy and 15 mg/100 mL for the food with peach soy. To establish the ideal quantity of RebA to be added in the beverages, we carried out tests with 15 non trained tasters, with formulations containing 1.5; 2.0; 2.5; 3.0; 3.5; 4.0 times more RebA than the sucralose quantities used in the formulations.

The formulations defined were: F1: Foods with peach soy with sucralose (13 mg/100 mL); F2: Foods with peach soy with RebA (39 mg/100 mL); F3: Foods with grape soy with sucralose (15 mg/100 mL); F4: Foods with grape soy with RebA (45 mg/100 mL).

Thus, the following analyzes were performed with these 4 pre-established formulations.

2.3 Physicochemical analysis

Total proteins and lipids

The quantification of total proteins in the grape soy food and the peach soy food was carried out at the laboratory of food and water of the chemistry department of UEM. The analysis was established according to the methodology by AOAC 16th Edition (Association of Official Analytical Chemists, 1995).

Total carbohydrates

The quantification of total carbohydrates of the grape soy food and the peach soy food was performed at the laboratory of food and water of the chemistry department at UEM. The analysis was determined following the methodology of total carbohydrates by phenol-sulfuric acid method of Dubois et al. (1956).

Soluble solids rate (°Brix) and pH

These parameters were measured according to the methodology by Instituto Adolfo Lutz (2005). All of the results were submitted to analysis of variance (ANOVA) followed by *Tukey* test ($p < 0.05$) and treated with *Statistical Analysis System software* (SAS) version 9.1.

2.4 Microbiological assessment

The microbiological analyses were carried out at the pharmaceutical sciences department of UEM according to the FDA/Bacteriological Analytical Manual methodology and recommendations of Brazilian legislation established by RDC nº12/2001 of ANVISA for flavored soy extract-based beverages posteriorly analyzed concerning coliforms at 45 °C, *Salmonella* spp and *Bacillus cereus* (Food and Drug Administration, 1995).

2.5 Sensory evaluations

The sensory tests involving tasters were conducted after approval of the Research Ethics Committee at UEM the project had been approved by the ethic committee of UEM, protocol 082/2009. The sensory assessment was conducted at the laboratory of sensory analysis of the chemistry department of UEM, with individual cabins and white light.

Both the foods with peach soy and with grape soy were used, both sweetened with RebA, compared with their sucralose version.

Seventy-five non trained tasters – both male and female of 19 to 50 years old – took part in the comparative tests; the samples were presented in different positions and with random repetitions. All tasters who agreed to participate in the sensory panel signed a consent form.

Along with the samples (approximately 25 mL) served in disposable white cups coded with random three-digit numbers, the taster was served with mineral water having been instructed to ingest it between samples to clean the palate in order to ensure the correct perception of the assessed aspects; they were also oriented to fill the evaluation form correctly.

In order to verify the tasters' acceptance and preference, we used a nine-point hedonic scale (1 = disliked strongly) to (9 = liked strongly) to assess the attributes of sweetness, residual taste (bitter), soy flavor and overall evaluation.

2.6 Statistical analysis

The results of all the physical-chemical analyses, sensory tests and physiological parameters were expressed as mean ± standard error of the mean (SEM) and submitted to analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) due to normal distribution. We used the *Statistical Analysis System software* (SAS) version 9.1 and *GraphPad Prism* 5.0® program.

3 Results and discussion

3.1 Equivalent sweetness

Different compositions at different concentrations of RebA were tested with the purpose of find one that had the sweetness equivalence of sucralose, the most common commercially available synthetic sweetener used in whey protein concentrates.

The concentration of RebA was established based on previous tests used in the composition of other products at NEPRON and in the literature (Carvalho et al., 2013). Fifteen trained tasters of both sexes, with ages between 20-55 years, specialized in evaluating the sweetness of sweeteners were invited to participate. The duo-trio test was used for sensory assessments (Dutcosky, 2013). The equivalent amount of sweetener necessary to provide the same sweetness as sucralose in the grape soy food and the peach soy food was calculated (Table 1). According to Table 1, it was observed that sucralose had the highest sweetness potency; however, an amount of RebA only three times was necessary to promote the same sweetness. Souza et al. (2013) showed the analysis of various sweeteners in low-sugar mixed fruit jam that the equivalent sweetness of sucralose/steviol glycoside was 2:1. Cardoso et al. (2004) found a higher amount of sweetness in stevia derivatives equivalence study of sweeteners and sweetening power consumption as a function of temperature in beverages containing tea mate soluble powder.

Table 1. Amount of added sweetener in the formulation.

Product	Sweetener (mg/100 mL)	
	Sucralose	RebA
Foods with peach soy	13	39
Foods with grape soy	15	45

RebA: rebaudioside A.

The assessed attributes were sweetness, residual flavor (bitter), soy flavor and overall evaluation with results indicated in Table 2. Sensory evaluation indicated significant difference ($p < 0.05$) for the attributes evaluated between food grape flavored soy and peach sweetened with sucralose and Reb A, showing that the tasters had better acceptance of both sweeteners in product peach flavor. The average scores given by the panelists were from "did not like so much" (6) and "liked moderately" (7). However, there were no significant differences promoted by the sweetness sweeteners, indicating that RebA can be used as a substitute for sucralose in the product without loss of sensory attributes.

3.2 Physicochemical assessment

Table 3 presents the results concerning the physicochemical analysis of the soy food sweetened with RebA with flavors of grape and peach formulated at the NEPRON, as well as the commercial food soy, sweetened with sucralose. The physicochemical analysis indicated no significantly differences ($p < 0.05$) for both formulations; therefore, the substitution of sucralose with RebA in the formulation does not affect the physicochemical properties of either the grape soy food or the peach soy food.

3.3 Microbiological assessment

Table 4 indicates the results of the microbiological analysis for the soy food sweetened with RebA grape and peach flavors. The result of the microbiological analysis indicated no contamination of the soy food during preparation since the product met the legal criteria and standards. This result is satisfactory since the microbiological standards are used to assess good manufacturing practices ensuring security.

Table 2. Sensory evaluation of food with soy grape and peach flavor sweetened with sucralose and sweetened with Reb A.

Evaluation	Grape		Peach	
	F1	F2	F3	F4
Sweetness	$6.96^{a} \pm 0.20$	$6.65^{a} \pm 0.18$	$7.16^{b} \pm 0.19$	$6.91^{b} \pm 0.17$
Residual taste (bitter)	$6.40^{a} \pm 0.23$	$6.27^{a} \pm 0.21$	$6.69^{b} \pm 0.22$	$6.72^{b} \pm 0.20$
Soy flavor	$6.95^{a} \pm 0.18$	$6.71^{a} \pm 0.18$	$6.83^{b} \pm 0.21$	$6.89^{b} \pm 0.20$
Overall evaluation	$6.95^{a} \pm 0.19$	$6.80^{a} \pm 0.16$	$7.31^{b} \pm 0.15$	$7.25^{b} \pm 0.15$

Data express mean ± SEM value. Significant differences between groups were analyzed by ANOVA analysis of variance. Different letters in the same line show significant difference at 5% probability level by Tukey test ($p < 0.05$). F1: Foods with peach soy with sucralose (13 mg/100 mL); F2: Foods with peach soy with RebA (39 mg/100 mL); F3: Foods with grape soy with sucralose (15 mg/100 mL); F4: Foods with grape soy with RebA (45 mg/100 mL). RebA: rebaudioside A.

Table 3. Physical-chemical analyzes of food with grape and peach soy.

Analyses	Grape		Peach	
	F1	F2	F3	F4
Soluble solids (°Brix)	3.30 ± 0.17[a]	3.50 ± 0.03[a]	3.00 ± 0.12[b]	3.10 ± 0.09 [b]
pH	3.70 ± 0.03[a]	3.80 ± 0.03[a]	3.80 ± 0.07 [b]	3.80 ± 0.06 [b]
Proteins	0.56 ± 0.01[a]	0.60 ± 0.01[a]	0.53 ± 0.01 [b]	0.50 ± 0.01 [b]
Lipids	0.26 ± 0.01[a]	0.30 ± 0.01[a]	0.25 ± 0.02 [b]	0.30 ± 0.01 [b]
Carbohydrates	1.98 ± 0.01[a]	2.05 ± 0.01[a]	1.20 ± 0.10 [b]	1.40 ± 0.10 [b]

Data express mean ± SEM value. Significant differences between groups were analyzed by ANOVA analysis of variance. Different letters in the same line show significant difference at 5% probability level by Tukey test ($p < 0.05$). F1: Foods with peach soy with sucralose (13 mg/100 mL); F2: Foods with peach soy with RebA (39 mg/100 mL); F3: Foods with grape soy with sucralose (15 mg/100 mL); F4: Foods with grape soy with RebA (45 mg/100 mL). RebA: rebaudioside A.

Table 4. Microbiological food rating with sweetened soy RebA.

Microorganisms	Products		Legislation
	Grape	Peach	RDC n°12/2001
Coliforms at 45 °C (MPN/mL)	< 3	< 3	10
Salmonella spp (25 mL)	-	-	-
Bacillus cereaus (CFU/mL)	< 10	< 10	5 x 10^2

RebA: rebaudioside A; CFU: colony-forming unit; MPN: most probable number.

4 Conclusion

It is concluded that high purity (97%) RebA produced at the Pilot's NEPRON using selected varieties is able to substitute sucralose in foods with soy peaches and grapes commercialized and patented by Cocamar (brand Purity) maintaining the same levels of quality for the assessed sensory parameters.

The new formulations were added to other products developed in our laboratories with the substitution of sucralose with Reb A, losing no sensory quality parameters, indicating that the Reb A is a potential substitute to replace sucralose and other sweeteners on a large scale of food products.

In addition, the formulations and analyzes obtained are important for the industry, especially those working with soy-based grape and peach flavored beverage formulations, because they contain high sales and consumption rates, proposing formulations sweetened with natural sweetener, with adjuvant properties in the treatment of metabolic diseases such as diabetes and obesity, in contrast to the most commonly accepted synthetic sweetener, without prejudice to taste and quality, and with good sensory acceptance.

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