

Replacing sugar with *S. rebaudiana* extracts on the physicochemical and sensory properties of strawberry ice cream

Substituição do açúcar com extratos de *S. rebaudiana* sobre as propriedades físico-químicas e sensoriais de sorvete de morango

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ABSTRACT

Ice cream is a product whose formulation requires considerable amounts of sugar. In addition to providing flavor, sugar contributes to the physicochemical characteristics of ice cream but its consumption in large quantities is related to chronic diseases such as diabetes and obesity. For this reason, the food industry seeks to formulate products with sweeteners that preserve the physicochemical and sensory characteristics of the original product. Stevia rebaudiana is a plant that naturally contains glycosides with no calories and high sweetening capacity and it is considered safe for consumption. Therefore the aim of this research was to evaluate the effect of replacing sugar with different levels of extracts of S. rebaudiana Morita II and Criolla, on the physicochemical and sensory properties of strawberry ice cream. Using a 2x2 factorial experimental design, a total of 4 formulations were prepared with two levels of concentration of the aqueous extract of Stevia rebaudiana (5 or 8%) and the variety of S. rebaudiana (Morita II or Criolla). Proximate composition, physicochemical properties and sensory evaluation were determined in processed products. The proximate analysis of strawberry ice cream varied significantly ($P < 0.05$) depending of variety and level used on the formulation. The viscosities of all ice cream mixes were decreasing as the shear rate was increased, indicating a pseudoplastic behavior. The sensory analysis showed differences ($P < 0.05$) among the formulations tested, however the score of all products were above the indifference point, suggesting that all of these formulations may have a commercial potential.

Key words: *Stevia rebaudiana*, ice cream, sweetener, proximate analysis, sensory evaluation.

RESUMO

Sorvete é um produto cuja formulação requer quantidades consideráveis de açúcar. Além de proporcionar o sabor, o açúcar contribui para as propriedades físico-químicas

do sorvete, mas o seu consumo em grandes quantidades está relacionada com as doenças crônicas, como diabetes e obesidade. Por essa razão, a indústria alimentícia buscou formular produtos com adoçantes para manter as propriedades físico-químicas e sensoriais do produto original. Stevia rebaudiana é uma planta que contém naturalmente glicosídeos sem calorias com alta capacidade adoçante, cujo consumo é considerado seguro. Portanto, o objetivo deste estudo foi avaliar o efeito da substituição do açúcar por diferentes níveis de extratos de S. rebaudiana Morita II e Criolla, sobre as propriedades físico-químicas e sensoriais do sorvete de morango. Usando um modelo experimental de fator 2x2, um total de 4 formulações foram preparadas com 2 níveis de concentração de extrato aquoso de Stevia rebaudiana (5 ou 8%) e a variedade de Stevia (Morita II ou Criolla). Os produtos processados foram submetidos a análises proximais e físico-químicas, e avaliação sensorial foi realizada para três deles. A análise do sorvete de morango teve uma variação estatisticamente significativa ($P < 0,05$), dependendo da variedade e do nível utilizado na formulação. A viscosidade da mistura de sorvete diminuía com o aumento da velocidade de corte, indicando um comportamento pseudoplástico. A análise sensorial mostrou que não houve diferenças significativas ($P > 0,05$) entre as formulações avaliadas e a pontuação de todos os produtos estava acima do ponto de indiferença, indicando que todos eles podem ter um potencial comercial.

Palavras-chave: *Stevia rebaudiana*, sorvete, adoçante, análise proximal de avaliação sensorial.

INTRODUCTION

“Ice cream is a food produced by freezing, while stirring, a pasteurized mix consisted of one or more of the optional dairy ingredients, and may contain

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one or more of the optional caseinates, subjected to the conditions herein after established, one or more of the optional hydrolyzed milk proteins, subjected to the conditions herein after the established, and other safe and suitable nonmilk-derived ingredients; and excluding other food fats, except those natural components of flavoring ingredients used or added in incidental amounts to accomplish specific functions. Ice cream is sweetened with safe and suitable sweeteners and may be characterized by the addition of flavoring ingredients” (FDA 2015). In the ice cream formulation, the main sweetener used is sucrose although other sugars can be used, such as glucose, fructose and sugar alcohols (CAILLET et al., 2003).

Higher intake of added sugars is associated with higher energy intake and lower diet quality, which can increase the risk for obesity, pre-diabetes, type-2 diabetes, and cardiovascular disease (KLURFELD, 2012). Therefore, the food industry has used alternatives to reduce the sugar content in their products, such as natural and artificial sweeteners (ACADEMY OF NUTRITION AND DIETETICS, 2012).

Stevia rebaudiana Bertoni is a good alternative, due to its high content of steviol glycosides which are natural, non-caloric and with a high sweetener capacity (200-300 times more than sugar) and are considered generally safe (GRASS) by the Food and Drug Administration (ACADEMY OF NUTRITION AND DIETETICS, 2012). Among the varieties of *S. rebaudiana*, Criolla and Morita II are the most used in the world. Criolla variety can be reproduced by cross-pollination (RAMÍREZ-JARAMILLO et al., 2011) allowing the producers to make empirical selections depending on weather conditions (LEMUS-MONDACA et al., 2012). Morita II variety was generated by a Japanese agronomist named Toyosigue Morita in order to generate a variety with higher content of Rebaudioside A and can be spread by seeds (MORITA & BU, 2000).

Extracts made from *Stevia rebaudiana* have many applications as sweeteners in the food industry, incorporated into a variety of products; however, eliminate sugar in the formulation of ice cream is not as simple as it may result in a product without adequate sensory characteristics (e.g. scoopability, mouthfeel, or even sweetness) (MARSHALL et al., 2003). Reducing sugar typically causes changes in the colligative properties of the ice cream (e.g. fraction of frozen water, and glass transition temperature) and the rheological properties due to the change in the size and shape of the crystals (SOUKOULIS et al., 2014; CAILLET et al., 2003). Therefore the aim of this research was to evaluate

the effect of replacing sugar with different levels of extracts of *S. rebaudiana* Morita II and Criolla, on the physicochemical and sensory properties of strawberry ice cream.

MATERIALS AND METHODS

Reagents and samples

Commercial products such as whole milk (Lala®), heavy cream (Alpura®) and frozen strawberries were purchased in a local market. The leaves of two varieties of *S. rebaudiana*, Morita and Criolla II were collected, dried, powdered and kept in the dark until use.

Stevia rebaudiana extracts

The leaves of two varieties of *S. rebaudiana*, Morita and Criolla II, were grown in the Southeast of Mexico. For preparing extracts, 0.5g of powdered leaves were weighed and extracted three times with water with 5mL each time in a boiling water bath at 100°C for 30min. Extracts were cooled to room temperature and centrifuged for 10 minutes (2,500 x g, 10°C) to facilitate separation of the aqueous phases of the leaves. The aqueous phases were transferred to a 25mL volumetric flask and filled to capacity after the last extraction (WÖELWER-RIECK et al., 2010).

Ice cream formulations

A 2x2 factorial experimental design was used to prepare the ice cream formulations. Factors and levels were: variety of *Stevia* (Criolla or Morita II) and concentration of extract used (5 or 8%) based on the original formulation. Four formulations (Table 1) were formed by combining different levels of each of the variables, performing two replicates

Table 1 - Strawberry ice cream formulations with *Stevia rebaudiana*.

Ingredients	Control	F1	F2	F3	F4
	-----Amount (% w/v)-----				
Whole milk	28.02	30.24	29.24	30.24	29.24
Strawberry	32.45	35.16	34.16	35.16	34.16
Heavy cream	27.02	29.3	28.3	29.3	28.3
CMC	0.3	0.3	0.3	0.3	0.3
Sugar	12.21	---	---	---	---
<i>Stevia rebaudiana</i>	---	5	8	5	8

Formulations prepared (F1: Morita II 5%, F2: Morita II 8%, F3: Criolla 5%, F4: Criolla 8%).

Values are based on 100 ml of ice cream mix.

of each formulation. Additionally a control group was prepared using sugar as sweetener. The base formulation was determined through preliminary tests developed in the laboratory.

Accordingly to formulation (Table 1), ingredients were weighed in the balance and placed in a blender to homogenize the mixture; they were liquefied until lumps disappear completely and then the mixture was subjected to whipping process in ice cream machine Taylor (previously frozen), where it underwent 5-7 minutes of mixing cold and 2min of aeration. At the end of this time, the mixture was stored at -18°C until analysis.

Proximate analysis

The proximate analysis of the four formulations was performed based on the methodologies proposed by the Association of Official Analytical Chemists (AOAC, 1997). Briefly: moisture (method 925.09) was determined by drying the sample in a convection oven at 110°C for 2 hours; ashes (method 923.03) were quantified by burning the sample at 550°C in a muffle; crude protein content (method 954.01) was estimated in a digester by Kjendahl method ($N \times 6.38$); crude fiber (method 962.09) was determined with a sequential acid and base digestion; fat was extracted by Soxhlet method for 80 minutes using hexane as the extraction solvent and Soxtec Foss apparatus at 120°C (method 920.39); and finally, carbohydrate content was established by difference and expressed as nitrogen-free extract.

Physicochemical analysis

To assess the quality of the ice cream, the following physicochemical parameters were determined:

Overrun (Constant Volume Method): Five hundred milliliters of the mixture were subjected to 5 minutes of mixing and then subjected to airy for two minutes in a Taylor machine (104-27 Illinois, USA); then the volume was measured and the overrun (%) was calculated, according to the equation $\text{volume of [(ice cream - volume of mix) / volume of mix} \times 100]$ (DERVISOGLU et al., 2005).

Mass of ice melted (Method Cottrell): The ice cream samples were weighed (20g) and placed on a wire mesh (1.15mm²); a previously weighed beaker was placed 10cm beneath the mesh to collect the melted ice cream. The system was kept at room temperature for 30min and then, the beaker with the melted ice cream was weighed and the mass of ice melted was calculated (COTTRELL et al., 1979). The assay was performed in duplicate for all samples.

Viscosity: The viscosity was determined as a percentage of torque and viscosity (Pa x s) in 8.6mL

of the ice cream mixture at 4°C. Brookfield DV-II+ Pro viscometer was used programmed in a shear rate range of 100-200 (1/s).

Sensory analysis

Among the four formulations of strawberry ice cream sweetened with *Stevia rebaudiana*, those products containing a higher concentration of aqueous extract of *Stevia rebaudiana* to provide a sufficient level of sweetness were selected by the formulator team to be tested. Since, *S. rebaudiana* Criolla contains more of stevioside and less rebaudioside A (ARANDA-GONZÁLEZ et al., 2014), the formulation F3 was discarded for sensory evaluation, as it was the less sweet product; therefore only F1, F2 and F4 were evaluated by a group of non-expert judges composed of 80 panelists.

The judges were of either sex, ranging in age between 20 and 50 years old, consisting of students, faculty and quartermaster staff of the Faculty of Chemical Engineering at the Universidad Autónoma de Yucatán. The judges noted the level of pleasure or displeasure through an unstructured hedonic scale of 10cm (Figure 1). The response variable was the level of satisfaction of products expressed in centimeters and was analyzed based on a statistical design with a completely random distribution.

Statistical analysis

The statistical analysis of proximate and physicochemical composition was performed by analysis of one-way variance; sensory analysis was subjected to non-parametric Kruskal-Wallis test. Differences were considered significant at $P < 0.05$ using Statgraphics plus software.

RESULTS AND DISCUSSION

The proximate composition of the different formulations of ice cream is given in table 2. As seen in table 2, those ice cream made with the same variety




Name of the product: <u>Strawberry ice cream</u>		
Name: _____	Date: _____	
Instructions: Test the product presented below, and indicate with a cross over the scale that best describes your opinion about the product you just tested		
Pleasantness		
Dislike very much	Neither like nor dislike	Like very much
		
Figure 1 - Unstructured scale. The line connecting the two ends of pleasantness has a known length of 10cm.		

Table 2 - Proximate composition of different formulation of ice cream sweetened with *S. rebaudiana* extracts (% dry basis).

Parameter (%)	F1	F2	F3	F4
Moisture	4.00 ^a	4.20 ^a	2.09 ^b	2.23 ^b
Ash	1.23 ^a	1.26 ^a	0.70 ^b	0.71 ^b
Crude fiber	1.02 ^{b,c}	1.32 ^a	0.94 ^c	1.06 ^b
Fat	23.02 ^{a,b}	22.41 ^b	23.82 ^a	22.63 ^b
Protein	5.51 ^a	5.69 ^a	2.48 ^c	2.86 ^b
Carbohydrate	65.22 ^c	65.12 ^c	69.97 ^b	70.51 ^a

Formulations prepared (F1: Morita II 5%, F2: Morita II 8%, F3: Criolla 5%, F4: Criolla 8%).

^{a-c}Different letters in rows indicate significant differences at $P < 0.05$.

of *Stevia rebaudiana* have a proximate analysis similarly to each other, but slightly different to the other variety. In other words, F1 and F2, which were made with *S. rebaudiana* Morita II, are similar but somewhat different from F3 and F4 that were made with *S. rebaudiana* Criolla. Comparison between formulations having the same amounts of ingredients (F1 and F2 to F3 with F4) was obtained statistical difference ($P < 0.05$) in moisture content, ash, fiber and carbohydrates respect to the variety of extract used. In the case of fat, no significant difference ($P > 0.05$) was reported. Generally, the component contents were higher when the extract of *Stevia rebaudiana* Morita variety was used.

In both varieties, the level of addition had an effect on proximal composition of ice cream; in Morita variety, level of addition increased the crude fiber with significant differences between formulations 1 and 2 ($P < 0.05$); in the Criolla variety, the formulation with more *Stevia* extract (F4) had higher content of crude fiber, protein and carbohydrates, while lower crude fat which was statistically different compared with F3 ($P < 0.05$). This is probably due to the differences between the chemical constituents present in the leaves of the varieties, as has been reported by SEGURA-CAMPOS et al. (2014).

The low humidity occasioned by the fruit solids used (by the use of frozen strawberries instead of syrup or marmalade), caused t a gritty texture (F3 and F4). It has been reported that solids in ice cream have an important role, as its lack made the structure weak but if they are in excess, the product will present a gritty texture (LÓPEZ-BARÓN et al., 2010). The ash content in F1 and F2, were similar and so did F3 and F4, accordingly to *Stevia* variety used. Varieties of *S. rebaudiana* cultivated in Mexico

contain high amounts of ashes ranging from 7.82-11.93% dry basis (SEGURA-CAMPOS et al., 2014). However, according to RAHMESH et al. (2006), growth conditions may affect the final content.

Regarding the fiber in the four formulations, the *Stevia rebaudiana* may provide it given that it is a good source of fiber (5.92-9.52% dry basis) according to SEGURA-CAMPOS et al. (2014) or simply from the fruit used in the formulation. As for the crude fat, the four treatments showed a great content, as the fat content in a standard ice cream formulation is about 12% (ARBUCKLE & MARSHALL, 2000). Given that any variety of *Stevia* contains fat, it is almost certain that is given by the milk and cream used in the formulation. Future formulations could consider using some fat replacers ingredients (e.g. inulin) (BARRIONUEVO et al., 2011), which would improve this aspect.

Apparent viscosity value of treatments to a strain rate of 10 1/s varied in a range of 0.062-0.324Pa x s at 23°C (Figure 2), however all four formulation had significantly lower viscosity compared to control (3.563Pa x s). This was due to the thickener and plasticizer effects of sucrose in solution at high concentrations. As shown in figure 2, the viscosities of all ice cream mixes studies were decreasing as the shear rate was increased, indicating a pseudoplastic or shear-thinning behavior. Also, the percentage of the extract increased viscosity statistically significant ($P < 0.05$); Viscosity of ice cream mix is affected by several factors, such as composition, kind and quality of ingredients, processing and handling of mix, total solids, and temperature (PATEL et al., 2011). Among the formulations made with *S. rebaudiana*, Morita II 8% (F2) showed the higher

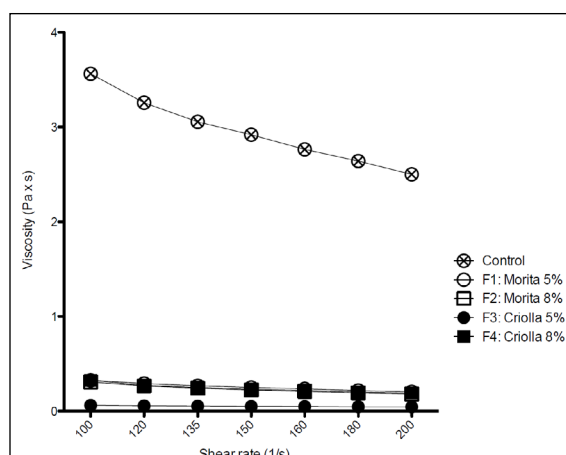


Figure 2 - Viscosity of different formulation of ice cream sweetened with *S. rebaudiana* extracts.

viscosity that could be caused by the amount of water (Table 2) and their ability to establish hydrogen bonds (PATEL et al., 2011).

Table 3 presents the results of measurements of melted ice cream and whipping performance with different substitution levels and varieties of *Stevia rebaudiana* extract used.

In the overrun test, it has been reported that when the apparent viscosity increases, the melt strength and softness of the body increases, but decreases performance whipping (ARBUCKLE & MARSHALL, 2000) which was observed in this study for ice cream obtained with sucrose respect to ice cream prepared with stevia extract. The ANOVA indicated that the stevia variety and extract concentration had significant influence ($P < 0.05$) on overrun, it was observed that the higher the concentration of extract, the lower the percentage of yield of whipping in both varieties.

In the test of melted mass, it was observed that in the control ice cream, the formulation with higher viscosity had lower melted mass (Table 2). The ANOVA data show that significant effect ($P < 0.05$) of stevia variety on melted mass. The ice cream sweetened with stevia Criolla extract showed less tendency to melt. The low or rapid melting is generally related to the amount of sweetener, stabilizer and/or emulsifier, therefore modifying this ingredient can modify such condition.

Among the four formulations, F1, F2 and F4 were subjected to sensory analysis; development group discarded formulation 3, due to its low sweetness. The average score awarded by the panelist are presented in figure 3 with significant differences ($P < 0.05$). More importantly, the ratings of the three evaluated formulations were above 5, which was above the point of indifference.

Table 3 - Physicochemical parameters of different formulation of ice cream sweetened with *S. rebaudiana* extracts.

Product	Overrun (%)	Melted mass (g)
Control	52.3 ^d	9.30 ^a
F1	64.0 ^{a,b}	4.01 ^b
F2	61.1 ^c	4.00 ^b
F3	65.2 ^a	3.24 ^c
F4	63.4 ^b	2.98 ^c

Formulations prepared (F1: Morita II 5%, F2: Morita II 8%, F3: Criolla 5%, F4: Criolla 8%).

^{a-d}Different letters in columns indicate significant differences at $P < 0.05$.

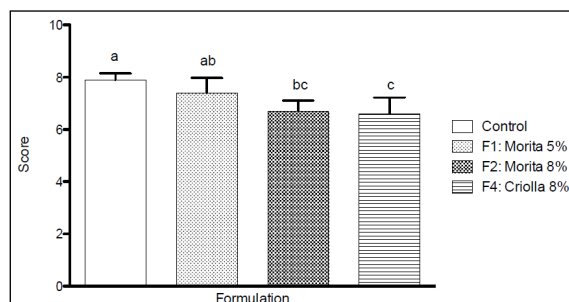


Figure 3 - Effect of *S. rebaudiana* addition on the sensory properties of strawberry flavored ice cream. The values represent the average score by the judges. Similar letters represent no significant differences

CONCLUSION

Four different formulations of ice cream sweetened with *Stevia rebaudiana* Morita II and Criolla were developed, whose proximate composition and physicochemical parameters were evaluated. Sensory evaluation showed significant differences between the products obtained, however all of them with a score above the indifference point suggesting that those products would have a potential to be commercialized.

ACKNOWLEDGEMENTS

This work was supported by Programa de Mejoramiento al Profesorado (PROMEP-SEP) and CONACYT's scholarship.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The authors of the article entitled "Replacing sugar with *S. rebaudiana* extracts on the physicochemical and sensory properties of strawberry ice cream" declared, for all due purposes, that the project that gave rise to the present data has not been submitted for evaluation to the Ethics Committee of the "Universidad Autónoma de Yucatán (Cuerpo Académico de Desarrollo Alimentario)", but we are aware of the contents of Resolution No. 466, of December 12, 2012 of the Brazilian National Health Council <http://conselho.saude.gov.br/resolucoes/2012/Reso466.pdf> if it involves human.

Thus, the authors assume full responsibility for the presented data and are available for possible questions that should be required by the competent authorities.

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