



## Trabajo Original

Obesidad y síndrome metabólico

### Development of nopal-pineapple marmalade formulated with stevia aqueous extract: effect on physicochemical properties, inhibition of $\alpha$ -amylase, and glycemic response *Desarrollo de mermelada de piña-nopal formulado con extracto acuoso de estevia: efecto sobre las propiedades fisicoquímicas, inhibición de $\alpha$ -amilasa y respuesta glicémica*

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#### Abstract

**Introduction:** *Stevia rebaudiana* extracts can be used as a sweetener due to their glycoside content: specifically stevioside and rebaudioside. Both compounds have adequate pharmacological characteristics for human consumption.

**Objective:** the aim of this study was to standardize the formulation of marmalades using nopal-pineapple-stevia aqueous extract ratios.

**Methods:** the products were evaluated to determine their physicochemical properties, *in vitro* inhibition of  $\alpha$ -amylase and glycemia in healthy volunteers. Storage study was conducted for 20 days at room temperature 23-30 °C and relative humidity 80-85%.

**Results:** incorporation of stevia significantly modified physicochemical properties like °Brix, color and flow index. After storage, the presence of molds and bacteria were not detected. Sensory evaluation indicated that marmalade with 50% stevia replacement was equally accepted as marmalade with sucrose. Marmalade with 50 and 100% of stevia inhibited 35.89 and 38.50% of the  $\alpha$ -amylase activity. After an intake of 30 g, it seems that marmalades with stevia had a significant effect on the glycemia of the volunteers.

**Conclusions:** however, further studies with larger doses of nopal-pineapple-stevia marmalade and consumed for longer in both healthy volunteers and patients with diabetes are needed to achieve results that are more precise.

#### Key words:

Nopal. Pineapple.  
Stevia. Marmalade.  
Glycemia.

#### Resumen

**Introducción:** los extractos de *Stevia rebaudiana* pueden ser utilizados como edulcorante debido a su contenido de glucósidos: específicamente esteviosido y rebaudiósido. Ambos compuestos presentan características farmacológicas adecuadas para el consumo humano.

**Objetivos:** el objetivo del presente trabajo fue estandarizar formulaciones de mermeladas con diferentes proporciones de nopal-piña-extracto acuoso de stevia.

**Métodos:** se estudiaron las propiedades fisicoquímicas de las mermeladas, su capacidad de inhibir *in vitro* a la enzima  $\alpha$ -amilasa y la glicemia en voluntarios sanos. Los estudios de vida de anaquel se efectuaron durante 20 días a temperatura ambiente 23-30 °C y humedad relativa 80-85%.

**Resultados:** la incorporación de estevia modificó significativamente los grados Brix, el color y el índice de flujo de las mermeladas. Concluido el estudio de anaquel, no se observó la presencia de hongos o bacterias. La evaluación sensorial indicó que la mermelada con 50% de estevia fue aceptada con el mismo nivel de agrado que la mermelada con sacarosa. Las mermeladas con 50 y 100% de estevia inhibieron la actividad de la  $\alpha$ -amilasa con valores de 35.89% y 38.50%, respectivamente. Posterior a una ingesta de 30 g de mermelada se observó un efecto significativo en la glicemia de voluntarios sanos.

**Conclusiones:** deberán efectuarse estudios de consumo prolongado y de mayores cantidades de mermelada tanto en voluntarios sanos como con diabetes para obtener resultados más precisos.

#### Palabras clave:

Nopal. Piña. Estevia.  
Mermelada. Glicemia.

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## INTRODUCTION

Diabetes mellitus is a complex chronic illness associated with a state of high blood glucose level, or hyperglycemia, occurring from deficiencies in insulin secretion, action, or both. This pathology affects more than 200 million people around the world. The World Health Organization (WHO) estimates that maintaining current morbidity the number of patients with diabetes will exceed 360 million by 2030 (1). Treatment methods for diabetes include dietary modifications and physical activity combined with specific drugs such as  $\alpha$ -glucosidase inhibitors, insulin, sulfonylurea, biguanide and troglitazone. However, side effects such as hypoglycemia and lactic acidosis have been reported with the use of pharmacological treatments (2).

Nopal (*Opuntia ficus-indica*) stems are widely known for its production of mucus. Studies suggested that nopal reduces absorption of water-soluble dietary fiber content by interrupting absorption of glucose in the intestine and showed reduced blood glucose levels after its ingestion (1,3). Pineapple (*Ananas comosus L.*) is known for possessing a wide array of pharmacological properties such as antibacterial activity, antihyperlipidemic activity, antitumor activity, antidiabetic and antioxidant. Some studies indicate that *Ananas comosus* possesses significant bioactivity, which in turn is partially due to the presence of antioxidant compounds (4).

*Stevia rebaudiana* is known as a calorie free bio sweetener of high quality and contains phytochemical compounds that helps to reduce blood sugar, cholesterol and blood pressure (5). Fifty grams of stevia leaf can replace 1,000 g of sugar, the sweetness of stevioside is non-fermenting, and it does not display browning while cooking (6). The impact of the *S. rebaudiana* aqueous extract on nutritional and sensory quality, its ability to reduce sugar intake and its antioxidant properties have been investigated (7).

The present study aims to standardize the formulation of the mixed marmalades using nopal-pineapple-stevia ratios and to study the physicochemical, inhibition of  $\alpha$ -amylase and glycemic response in healthy volunteers after ingestion.

## MATERIALS AND METHODS

### VEGETAL MATERIAL AND CHEMICALS

*Stevia rebaudiana* (Bertoni) var. Morita II was obtained from plots established in Yucatan, México. Samples were obtained from the first cut of the plot at an age of three months. All chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### S. REBAUDIANA AQUEOUS EXTRACT PREPARATION

*S. rebaudiana* leaves were subjected to convection drying at 60 °C for 24 h. The leaves were milled to obtain particles of

1.0 mm in size. The extract was prepared by mixing one part of stevia leaves with nine parts of water. Water was heated to 55 °C and then mixed with stevia powder. The mixture was allowed to steep for one hour. After cooling, the extract was filtered and centrifuged at 3,000 rpm for 15 minutes. The extract was stored in amber bottles at 4 °C until analysis.

### PREPARATION OF NOPAL-PINEAPPLE-STEVIA MARMALADE

The preparation of mixed fruit marmalade was done as follows. All the required ingredients were weighed correctly to obtain three formulations (Table I). The nopal leaves were sanitized and the thorns were removed, then the leaves were cut into small pieces and bleached in water at 100 °C for ten minutes. The leaves were submerged in ice water to stop cooking and finally crushed in a food processor. On the other hand, the pineapple was sanitized and the leaves and skin were removed. The pulp was cut into small pieces and crushed in a food processor. Sucrose, stevia aqueous extract, and pectin were mixed thoroughly, and then processed fruit was added and cooked at 95 °C during 45 minutes. Finally, lemon juice was added as an acidifier. The marmalades were allowed to cool for 24 hours and became jellified in the glass jars. The jars were capped properly and stored at room temperature. All measurements were carried out in triplicate for each batch on the first day of storage and after five, ten, 15, and 20 days.

### pH, WATER ACTIVITY AND SOLUBLE SOLIDS CONTENT

pH was measured using a pH-meter. Water activity ( $a_w$ ) was determined using a hygrometer at 25 °C. Soluble solids content of samples were measured by a refractometer at 20 °C.

**Table I.** Marmalade formulations: nopal-pineapple marmalade with sucrose (NPSu), nopal-pineapple marmalade with sucrose and stevia (NPSuSt) and nopal-pineapple marmalade with stevia (NPSt)

Ingredient (%)	NPSu	NPSuSt	NPSt
Nopal	38.31	47.03	50.12
Pineapple	44.29	40.68	43.35
Pectin	0.55	0.58	0.62
Lemon juice	1.32	1.41	1.50
Sucrose	15.50	8.23	0.0
Stevia extract	0.0	2.05	4.38

## OPTICAL PROPERTIES

Optical properties were measured using a spectrophotometer. CIE  $L^*a^*b^*$  coordinates were obtained and total color difference was calculated using the following formula:  $\Delta E = [(L2^* - L1^*)^2 + (a2^* - a1^*)^2 + (b2^* - b1^*)^2]^{1/2}$  (8).

## VISCOSITY MEASUREMENT AND RHEOLOGICAL BEHAVIOR

Viscosity (Pa·s) was measured at 25 °C using a Brookfield rotational viscometer equipped with spindle no. 28 at a speed of 10 to 200 rpm. Enough marmalade in a 500-ml beaker was used to immerse the groove on the spindle with the guard leg. Three readings were taken per sample at 30-second intervals. The empirical data obtained for samples were converted into shear stress and shear rate. Average shear stress and shear rate were calculated as:

$$\sigma_a = k_{\alpha\sigma} \cdot C \text{ (value read from the viscometer) (1)}$$

$$\gamma_a = k_{N\gamma} \cdot N \text{ (2)}$$

Where  $\sigma_a$  is the average shear stress (Pa),  $k_{\alpha\sigma}$  is the shear stress conversion factor (Pa) and C is the spring constant (C = 1.0). N is the rotational speed in rpm.

Dividing Eq. 1 by Eq. 2 yields an expression for apparent viscosity:

$$\eta_a = \sigma_a / \gamma_a \text{ (3)}$$

The rheological behavior of marmalades is described by the power law model.

$$h_a = k \cdot \gamma^{(n-1)} \text{ (4)}$$

Where  $h_a$  is the apparent viscosity (Pa·s),  $k$  is the consistency index (Pa·s<sup>n</sup>),  $\gamma$  is the shear rate (s<sup>-1</sup>) and  $n$  is the flow behavior index. Linear regression analysis was applied on the data to find  $n$ ,  $k$  and correlation coefficient.

## MICROBIOLOGICAL STUDY COUNTING OF YEAST AND MOULD

Yeasts, moulds, and mesophilic aerobic bacteria were determined on the first day of storage and after five, ten, 15, and 20 days (9,10).

## SENSORY EVALUATION

The panel consisted of 80 male and female, aged 18 to 35, non-smoker panelists. For evaluation, approximately 30 g of each marmalade sample were given to assessors labelled with random three-digit codes. The samples were brought to room temperature before testing, and served under white lightning in porcelain plates. Each panelist received a rating form, a slice of white bread, and a knife for each blend. At each session, sensory attributes were discussed. After palate cleaning, a pause (15 s) was imposed before the panelists could assess the next sample. The panelists

were asked to rate their acceptability for the product on a hedonic scale, 1 to 7, ranging from "dislike extremely" to "like extremely". Some numerical values are assigned to each point on the scale to analyze the result using statistical methods. The results were evaluated by analysis of variance and Duncan multiple range test (11).

## $\alpha$ -AMYLASE INHIBITORY ASSAY

The assay was carried out following the standard protocol with slight modifications (12). Starch (2 mg) was suspended in a tube containing 0.2 ml of 0.5 M Tris-HCl buffer (pH 6.9) with 0.01 M calcium chloride (substrate). The tube was boiled for five minutes and then incubated at 37 °C for five minutes. Stevia aqueous extract and samples (10 mg/ml) were dissolved with 1 ml of 0.1% of dimethyl sulfoxide in order to obtain concentrations of 50, 100, 200, 400, 600, 800 and 1,000  $\mu$ g/ml. Then 0.2 ml of stevia aqueous extract of a particular concentration was put in the tube containing the substrate solution. Then, 0.1 ml of porcine pancreatic amylase in Tris-HCl buffer (2 units/ml) was added to the tube containing stevia aqueous extract and starch. The process was carried out at 37 °C for ten minutes. The reaction was stopped by adding 0.5 ml of 50% acetic acid to each tube. The reaction mixture was then centrifuged at 2,000 x  $g$  for five minutes at 4 °C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer. The assay was performed in triplicate. The  $\alpha$ -amylase inhibitory activity was calculated as follows:

$$\alpha\text{-amylase inhibitory activity} = \frac{(AC^+) - (AC^-) - (AS - AB)}{(AC^+) - (AC^-)} \times 100$$

Where  $AC^+$ ,  $AC^-$ , AS and AB are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively.

## EFFECT OF INTAKE ON BLOOD GLUCOSE LEVELS

The effect on blood glucose levels after an intake 50 g of marmalades with sucrose and replaced with 50 and 100% of stevia aqueous extract was evaluated in ten healthy non-diabetic volunteers. Characteristics of the volunteers: five female and five male, average age of  $29 \pm 6.1$  years, height of  $163.5 \pm 3.1$  cm, weight of  $53.4 \pm 2.3$  kg and body mass index (BMI) of 20.75. The measurement of the serum glucose levels of the volunteers was determined with a glucometer. After the measurement of fasting glucose levels, the volunteers consumed 50 g of marmalade and glucose levels were measured after two and four hours, respectively. Volunteers had two days of a washout period before another marmalade testing. This study was conducted with the approval of the committee of Universidad Autónoma de Yucatán in accordance with the concept of the Declaration of Helsinki, and written consent of all volunteers was obtained after an explanation of the contents and method of study.

## STATISTICAL ANALYSIS

Descriptive statistics were obtained for all studied variables. Results were presented as mean  $\pm$  standard deviation (SD). Differences between two treatment groups were analyzed using an independent t-test. The repeated measures ANOVA were used to compare the differences within formulations or groups. All p values less than 0.05 were considered as statistically significant.

## RESULTS

The pH values oscillated between 3.56 and 3.83 over time (Table II). For all formulations, it was observed that the pH increases during the first five days of storage. By day 10, the pH returns to values close to the start of the study. Finally, by day 15 the values rise again reaching maximums at day 20. Formulations registered significant differences of water activity (Table II), with values between 0.938 and 0.957 at the end of the study. The results indicate that both the incorporation of the aqueous extract of stevia and the storage time influenced the behavior of the water activity. The content of soluble solids was reduced as the addition of aqueous stevia extract increased; final contents oscillated between 11.27 and 35.27% (Table II).

The color index is one of the most important factors in the quality of fruit products such as marmalades produced by heat treatment. Manufacturing processes such as dilution, drying and baking can affect the final product color. Significant differences were found in color values of all three types of marmalades. Table III shows

the effect of stevia incorporation on optical properties (color) of marmalades.

Marmalades produced with aqueous extract of stevia had the highest lightness/brightness values ( $L = 48.24$ ), whereas marmalade formulated with sucrose + aqueous extract of stevia had the lowest L values (40.28). After 20 days, the greenness/redness values (a) of the products ranged from 3.21 to 5.27 and blueness/yellowness values (b) ranged from 23.82 to 28.98. Total color difference values ranged from 52.36 to 60.38.

One way to understand changes in the structure of foods during processing is to study the rheological parameters. To determine the flow behavior of the marmalades, the power law model was used, with the objective of obtaining the values of the consistency index ( $k$ ) and the flow behavior index ( $n$ ). These values are shown in table IV. During the storage, the consistency index was modified, showing a significant reduction at 20 days.

$k$  values indicate that marmalade formulated with sucrose is more fluid, and has more homogeneous texture and uniform particle distribution than marmalades formulated with aqueous extract of stevia.

Microbiological study counting of yeast and moulds by standard plate count method and count was expressed as cfu/g of sample. The total no. of viable bacteria, yeast and moulds were counted multiplying the colony-forming unit (cfu) with dilution number. Samples showed minimum viable count for mesophiles ( $< 5.0 \log \text{cfu/g}$ ), coliforms ( $< 1.0 \log \text{cfu/g}$ ), yeast and moulds ( $< 2.0 \log \text{cfu/g}$ ), salmonella (negative en 25 g) and *Escherichia coli* (negative in 1 g), after 20 days of study.

The overall acceptability revealed that marmalade formulated with 50% sucrose + 50% of stevia aqueous extract was more

**Table II. Effect of stevia incorporation on physicochemical properties**

Time	pH			$a_w$			°Brix		
	NPSu	NPSuSt	NPSt	NPSu	NPSuSt	NPSt	NPSu	NPSuSt	NPSt
0	3.70 <sup>†</sup>	3.63 <sup>*</sup>	3.65 <sup>*</sup>	0.923 <sup>*</sup>	0.941 <sup>*</sup>	0.953 <sup>*</sup>	37.17 <sup>†</sup>	26.00 <sup>*</sup>	11.83 <sup>*</sup>
5	3.75 <sup>†</sup>	3.71 <sup>†</sup>	3.76 <sup>†</sup>	0.937 <sup>†</sup>	0.944 <sup>†</sup>	0.960 <sup>†</sup>	36.33 <sup>†,§</sup>	26.00 <sup>*</sup>	12.67 <sup>§</sup>
10	3.60 <sup>*</sup>	3.56 <sup>*</sup>	3.66 <sup>*</sup>	0.932 <sup>†,‡</sup>	0.941 <sup>*</sup>	0.956 <sup>*</sup>	36.23 <sup>*,†,‡</sup>	26.17 <sup>*</sup>	12.00 <sup>†</sup>
15	3.68 <sup>†</sup>	3.69 <sup>†</sup>	3.73 <sup>†</sup>	0.927 <sup>*,†</sup>	0.944 <sup>†</sup>	0.951 <sup>*</sup>	35.80 <sup>*,†</sup>	26.10 <sup>*</sup>	11.93 <sup>†</sup>
20	3.82 <sup>§</sup>	3.80 <sup>†</sup>	3.83 <sup>†</sup>	0.938 <sup>†</sup>	0.957 <sup>†</sup>	0.957 <sup>†</sup>	35.27 <sup>*</sup>	25.87 <sup>*</sup>	11.27 <sup>*</sup>

Different superscripts indicate significant statistical difference.  $a_w$  = water activity; NPSu: nopal-pineapple marmalade with sucrose; NPSuSt: nopal-pineapple marmalade with sucrose and stevia; NPSt: nopal-pineapple marmalade with stevia.

**Table III. Effect of stevia incorporation on optical properties**

Time (days)	NPSu				NPSuSt				NPSt			
	L*	a*	b*	$\Delta E$	L*	a*	b*	$\Delta E$	L*	a*	b*	$\Delta E$
0	43.56 <sup>†</sup>	6.91 <sup>†</sup>	30.52 <sup>†</sup>	59.87 <sup>†,‡</sup>	44.23 <sup>†</sup>	4.31 <sup>§</sup>	24.49 <sup>†</sup>	55.74 <sup>§</sup>	47.46 <sup>†</sup>	5.05 <sup>†</sup>	29.21 <sup>†</sup>	55.35 <sup>†</sup>
5	41.47 <sup>†</sup>	5.67 <sup>†,‡</sup>	20.82 <sup>†,§</sup>	56.89 <sup>§</sup>	43.58 <sup>†</sup>	5.41 <sup>†</sup>	20.73 <sup>§</sup>	55.64 <sup>§</sup>	42.94 <sup>†,‡</sup>	4.55 <sup>†,§</sup>	21.91 <sup>†,§</sup>	55.67 <sup>†</sup>
10	37.60 <sup>†</sup>	4.98 <sup>†</sup>	18.55 <sup>§</sup>	59.68 <sup>†</sup>	45.38 <sup>†</sup>	5.22 <sup>†,§</sup>	28.35 <sup>†</sup>	56.56 <sup>†</sup>	43.51 <sup>†</sup>	3.81 <sup>†,§</sup>	20.02 <sup>§</sup>	54.54 <sup>†</sup>
15	42.03 <sup>†</sup>	3.15 <sup>§</sup>	24.23 <sup>†,‡</sup>	57.45 <sup>§</sup>	47.59 <sup>†</sup>	5.86 <sup>†</sup>	27.14 <sup>†,‡</sup>	54.13 <sup>†</sup>	47.44 <sup>†</sup>	2.91 <sup>§</sup>	29.07 <sup>†</sup>	54.94 <sup>†</sup>
20	41.28 <sup>†,‡</sup>	4.93 <sup>†</sup>	28.98 <sup>†,‡</sup>	60.38 <sup>†</sup>	40.28 <sup>§</sup>	5.27 <sup>†,§</sup>	23.82 <sup>†,§</sup>	59.07 <sup>†</sup>	48.24 <sup>†</sup>	3.21 <sup>§</sup>	25.23 <sup>†</sup>	52.36 <sup>†</sup>

Different superscripts indicate significant statistical difference. L\* = lightness, a\* = red/green, b\* = yellow/blue,  $\Delta E$  = total color difference. NPSu: nopal-pineapple marmalade with sucrose; NPSuSt: nopal-pineapple marmalade with sucrose and stevia; NPSt: nopal-pineapple marmalade with stevia.

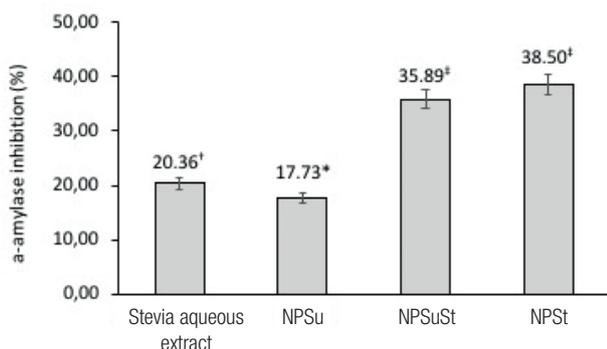
**Table IV.** Effect of stevia incorporation on consistency index (*k*) and flow behavior index (*n*)

Time (days)	Consistency index ( <i>k</i> )			Flow behavior index ( <i>n</i> )		
	NPSu	NPSuSt	NPSt	NPSu	NPSuSt	NPSt
0	3.117*	3.757*	4.748 <sup>†</sup>	0.041 <sup>†</sup>	0.029 <sup>†</sup>	0.253 <sup>§</sup>
5	5.483 <sup>‡</sup>	6.987 <sup>§</sup>	7.036 <sup>§</sup>	0.015*	0.011*	0.036 <sup>‡</sup>
10	5.627 <sup>§</sup>	5.056 <sup>‡</sup>	6.882 <sup>‡</sup>	0.039 <sup>†</sup>	0.022 <sup>†</sup>	0.009*
15	5.103 <sup>‡</sup>	4.312 <sup>†</sup>	6.148 <sup>‡</sup>	0.053 <sup>‡</sup>	0.129 <sup>§</sup>	0.033 <sup>‡</sup>
20	3.899 <sup>†</sup>	3.918*	3.725*	0.072 <sup>§</sup>	0.052 <sup>‡</sup>	0.015 <sup>†</sup>

Different superscripts indicate significant statistical difference. NPSu: nopal-pineapple marmalade with sucrose; NPSuSt: nopal-pineapple marmalade with sucrose and stevia; NPSt: nopal-pineapple marmalade with stevia.

preferred due to its highest mean score (5.06), score that does not differ from that obtained by the marmalade formulated with 100% sucrose (4.92). Of the three samples, marmalade formulated with 100% of stevia aqueous extract was inferior due to its low mean score (3.79). This can be attributed to the taste of stevia remains in mouth after marmalade consumption.

$\alpha$ -amylase had been recognized as therapeutic target for the modulation of postprandial hyperglycemia. Postprandial hyperglycemia is the earliest metabolic abnormality to occur in type 2 diabetes mellitus. Studies suggested that  $\alpha$ -amylase and inhibitors could lengthen the duration time of carbohydrate absorption and flatten the concentration of the blood glucose curve over time (13). The inhibitory activity of stevia aqueous extract and marmalades was evaluated by employing, in separate experiments, the samples as substrates of the enzymatic reaction. The results show the efficiency of the extract and marmalades in reducing polysaccharide digestion and, thus, glucose absorption. Figure 1 shows the percentage of  $\alpha$ -amylase inhibition of the stevia aqueous extract and different marmalade formulations.

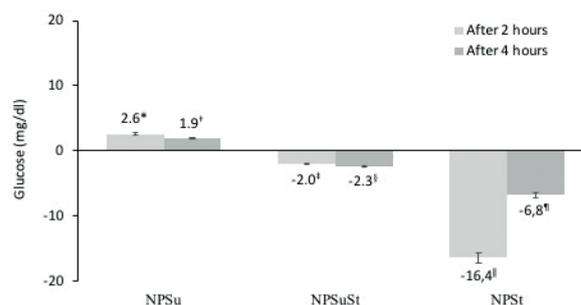


**Figure 1.** Effect of stevia incorporation on  $\alpha$ -amylase inhibition of marmalades (NPSu: nopal-pineapple marmalade with sucrose; NPSuSt: nopal-pineapple marmalade with sucrose and stevia; NPSt: nopal-pineapple marmalade with stevia. Different superscripts indicate significant statistical difference).

The lowest percentage of inhibition corresponds to the marmalade sweetened with sucrose (17.73), however, it is interesting that it has inhibitory activity. This activity could be attributed to bioactive compounds naturally present in nopal and pineapple. Stevia extract had the ability to inhibit 20.36% of the enzymatic activity. The marmalades formulated with 50% and 100% of stevia did not present significant differences in their capacity to inhibit the enzymatic activity.

The effect of the intake of the three marmalade formulations on the blood glucose levels of healthy volunteers was determined (Fig. 2). The intake of nopal-pineapple marmalade with sucrose significantly reduced the blood glucose level of four volunteers, maintaining the effect four hours later. The rest of the volunteers did not show changes or their blood glucose levels rose above their fasting levels.

Ingestion of both marmalades with stevia had a significant effect on the glycemia of the volunteers. This effect is more evident in the marmalade formulated with stevia (100%), since after four hours of intake, glucose levels were observed below those that were determined in fasting (Fig. 2).



**Figure 2.** Effect of marmalade ingestion on glycemic response of healthy volunteers (*n* = 10). Each value expressed as mean  $\pm$  SE of volunteers (NPSu: nopal-pineapple marmalade with sucrose; NPSuSt: nopal-pineapple marmalade with sucrose and stevia; NPSt: nopal-pineapple marmalade with stevia. Different superscripts indicate significant statistical difference).

## DISCUSSION

The pH is an important parameter that indicates the quality of the marmalade, thus establishing the guidelines for the approach of strategies of conservation of the product. Although the data show significant differences ( $p < 0.05$ ) in pH over time, the final values are below the 4.5 considered as critical for microbiological safety of marmalades (14). The  $a_w$  values of marmalades are higher than 0.9, which would make them susceptible to the proliferation of yeasts, moulds and bacteria. Although the high concentration of sucrose in conventional marmalade, acid pH, and the preservation and packaging techniques prevent microbial growth, in marmalades formulated with stevia it would be necessary to incorporate an antimicrobial additive. The content of soluble solids was reduced as sucrose was replaced by aqueous extract of stevia.

According to the color determinations, the product with the best characteristics of luminosity and more defined tonalities is the marmalade formulated with stevia extract. For rheological behavior, the values of the flow behavior index ( $n$ ) in three formulations was less than 1, suggesting the behavior of a pseudoplastic fluid, in which viscosity decreases with increasing cutting speed (15). The values of  $n$  are similar to those obtained using low pectin concentrations (less than 1% in this study). Studies of microbiological stability showed minimum viable count for mesophiles, coliforms, yeast, and moulds. The presence of salmonella and *Escherichia coli* was negative. For sensory evaluation, marmalade formulated with 100% of stevia aqueous extract had the least acceptance due to the bitter aftertaste of stevia.

Results for studies of  $\alpha$ -amylase inhibitory activity indicate that the inhibitory activity of marmalades results from the synergism of the biological activity of the compounds present in their ingredients: nopal, pineapple and stevia extract. The effect of the different nopal-pineapple marmalades formulations on the reduction of the glucose levels of volunteers could be due to the presence of compounds with biological activity capable of affecting the metabolism of glucose. In this sense, nopal reduced absorption of water-soluble dietary fiber content by interrupting absorption of glucose in the intestine and showed reduced blood glucose levels after ingestion (1). Studies have shown that bioactive compounds of pineapple like sinapic acid, daucosterol, 2-methylpropanoate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, methyl 2-methylbutanoate and triterpenoid ergosterol have antioxidant potential, anti-glycation potential and inhibited carbohydrate digestive enzymes (16). In the case of stevia, some of its components like stevioside, rebaudioside A and their aglycon steviol potentiate the activity of TRPM5 a  $Ca^{2+}$ -activated cation channel expressed in type II taste receptor cells and pancreatic  $\beta$ -cells. Steviol glycosides enhance glucose-induced insulin secretion in a Trpm5-dependent manner. Consumption of steviol glycosides could prevent development of hyperglycemia (17).

## CONCLUSIONS

The results indicate that the nopal-pineapple marmalades formulated with aqueous extract of stevia exhibit adequate physico-

chemical and optical properties, as well as a rheological behavior typical of a marmalade with low pectin content. Sensory evaluation indicated that marmalade with 50% stevia replacement was equally accepted as marmalade with sucrose. Nopal-pineapple marmalades inhibited *in vitro* the  $\alpha$ -amylase activity. Its ingestion had a significant effect on the glycemia of the volunteers. This effect is more evident in the marmalade formulated with stevia (100%), since after four hours of intake, glucose levels were observed below those that were determined in fasting. However, further studies with larger doses of nopal-pineapple-stevia marmalade and consumed for longer in both healthy volunteers and patients with diabetes are needed to achieve more precise results.

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