Development of a snack from xoconostle (*Opuntia matudae* Scheinvar) sweetened with neotame and its antioxidant capacity

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ABSTRACT

The xoconostle (*Opuntia matudae* Scheinvar) is a sour fruit, of which the peel is consumed. It is perishable, but it has a beneficial effect on human health, as recognized popularly in Mexico. The objective of this study was to create a snack of dried xoconostle using neotame, an artificial non-caloric sweetener, to improve flavor. We present (a) a partial chemical characterization of the fruit (total phenolic content (TP) and antioxidant activity (AA)), (b) dehydration techniques (heating by convection and freeze-drying), and (c) sensorial characterization of the final product. The experimental unit was 40 g of fresh xoconostle peel with three replications. The sweetener did not affect dehydration time of convective heat. The sweetened lyophilized xoconostle had lower TP and AA than unsweetened xoconostle. The sensorial analysis showed that lyophilized xoconostle sweetened with neotame (aqueous solution 32 mg L^{-1}) has acceptable flavor and appearance.

Keywords: dehydration, non-caloric sweeteners, sensorial evaluation, total phenolic, freeze drying

INTRODUCTION

Fruits provide nutrients, and they can prevent some diseases because of chemical substances that can modify organism physiology. These substances are non-nutritive compounds, classified as secondary metabolites, also known as phytochemicals with bioactive properties (Torres and Tovar-Palacios, 2009). This is the case of antioxidant compounds such as vitamin C, carotenoids, phenols and flavonoids, which, due to their chemical nature, neutralize free radicals (chemically unstable and reactive molecules). In

human tissues, free radicals are formed continuously as a subproduct of respiration and lipid metabolism (Frei, 1994).

The xoconostle (*Opuntia matudae* S.) is an edible cactus fruit; it is sour, with a pH that varies between 2.71 and 3.42 (Scheinvar *et al.,* 2009). It is used in traditional medicinal remedies (Guzmán *et al.,* 2009), in food as flavoring in some typical Mexican dishes, and as raw material for brewing liqueurs, and preparing jams, jellies, preserves and sauces (Olivares *et al.,* 2003; Scheinvar *et al.,* 2009).

Guzmán-Maldonado *et al.* (2010) analyzed the chemical composition of xoconostle fruits and pointed out that increasing intake of xoconostle can contribute to the prevention of some chronic diseases. Pimienta-Barrios *et al.* (2008) concluded that regular consumption of fruit peel of xoconostle (O. *joconostle*) can help control serum glucose in individuals with type 2 diabetes, and in healthy individuals its consumption can prevent hyperglycemia and concentration of cholesterol and triglycerides. Among the common people, xoconostle is regarded as beneficial for good health, but consumption is low because the peel, which is the tissue that is eaten (Álvarez and Peña-Valdivia, 2009), is sour and not sweet. With the aim of increasing consumption of xoconostle, the possibility of creating candy from xoconostle, sweetened with neotame was posed. Neotame is an artificial sweetener derived from aspartame; it is 6 000 to 10 000 times sweeter than sucrose but non-caloric, nontoxic and helps avoid problems such as tooth cavities and others associated with excessive consumption of caloric sweeteners (Nofre and Tinti, 2000).

Like other fruits, xoconostle deteriorates after harvest. Among the methods of food preservation, drying or dehydration is used to almost entirely to diminish the metabolic activities of tissues and to prevent invasion of microorganisms (Bello, 2000). Methods of dehydration are varied, and selection of the method depends on the type of material to be dehydrated. Fruit tissues can be dehydrated by convective heat or freeze-drying, among other methods. The objective of this study was to develop a snack from dehydrated xoconostle and to improve its flavor with neotame.

MATERIALS AND METHODS

Plant material

Two hundred and fifty xoconostle fruits (*Opuntia matudae* Scheinvar) cv. Cuaresmeño were collected from a family garden in the community of Cuautlacingo, Municipality of Otumba, State of Mexico. The fruits were harvested at commercial maturity stage (uniform size and peel colors) free of pests and diseases.

Effect of sweetening time on drying by hot air convection

Fruits were disinfected externally by immersion in an aqueous solution of 0.01 % sodium hypochlorite; excess solution was eliminated by draining on absorbent paper; the peel was

then removed with a stainless steel peeler, and seeds were eliminated. From the peel, slices 3 ± 0.1 mm thick were obtained.

To select sweetening time, slices were placed in an aqueous solution of neotame (11 mg L^{-1} w/v), in a 1:1.5 (g: mL) proportion of peel: solution for 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 h. Neotame was not added to one batch (control sample). Excess solution was then drained off for 10 min. The samples of the seven treatments were placed in the trays of a hot air drier at 55 ºC and air speed at exit of 1.2 m s^{-1} ± 0.1. The mass (g) of the sample and the aqueous activity (a_w) were measured in the sample extracted from the dehydrator at the beginning and every 10 min for 7 h; mass was measured with a digital scale (TP4KD Ohaus, USA) attached to the dehydrator, and a_w was measured with an aqueous activity meter (AquaLab, Decagon Devices, Washington, USA), and the average of two readings from each sample was obtained.

Total dry-base moisture (g H_2O g^{-1} dry solid) was quantified by the method described by Geankoplis (1998). Moisture in the solid was calculated with equation: $X_t = m_w / m_{Sd}$, where X_t is the total dry-base moisture (g H_2O g^{-1} dry solid) for each time, m_w is the water mass (g) for each time and m_{Sd} is the solid dry mass (g) constant value. With the data obtained periodically, drying curves were obtained from the total dry base moisture (g H_2O g^{-1} dry solid) of the peel in function of time (h) (Fito *et al.,* 2001).

Effect of neotame concentration on freeze-drying

Xoconostle slices were immersed in aqueous solutions of neotame with concentrations of 11, 22, 33, 44 and 55 mg L^{-1} (w/v), 1:1.5 (g:mL) peel:solution, during 1 h. One batch was not sweetened (control sample). Excess solution was drained for 10 min. The samples were placed in (20 X 10 cm) yute bags and frozen in a horizontal freezer (FFC0923DW Frigidaire, USA) for 12 h at -20 ºC. They were then dehydrated in a freeze-dryer (7670520 LABCONCO, Kansas, USA) at -40 °C and 12 Pa, during 12 h. Mass and a_w were assessed at the beginning of the process and every hour during 12 h, as described above. To weigh the sample, each bag was removed from the recipient and placed on an analytical scale (Scout Pro: SPE123 Ohaus, USA); mass was recorded in grams; a_w was measured with an aqueous activity meter (AquaLab, Decagon Devices, Washington, USA), and the average of two readings of each sample was obtained.

Quantification of total phenolic content

Total phenolic content was quantified by the Folin-Ciocalteu´s colorimetric method described by Waterman and Mole (1994), with gallic acid as the standard. Samples of 1 g of freeze-dried peel were immersed in 25 mL of 95 % (v/v) aqueous ethanol and were sonicated for 20 min in an ultrasonic bath (Cole-Parmer, Pasadena, USA). The supernatant was collected, and 80 % (v/v) aqueous ethanol was added to obtain the extract and adjust the final volume to 25 mL. Aliquots of 0.5 mL of the extracts were mixed with 10 mL of a solution of anhydrous sodium carbonate (100 g L^{-1}), and the mixture was incubated during 15 min at 38 ºC. After, 1 mL of the mixture was mixed with 3 mL of water and 1 mL of Folin-Ciocalteu´s reactive (previously diluted with water 1:1 v/v). The samples were shaken and allowed to stand during

15 min in the dark at room temperature. Absorbance was then measured at 660 nm with a spectrophotometer (Spectronic 20®, Florida, USA). The equation fit by linear regression of the standard curve was y = 0.000212 x – 0.0142 (R^2 = 0.995). The total phenolic content was expressed in mg of gallic acid equivalents / 100 g dry matter (mg GAE / 100 DM).

Antioxidant activity

Antioxidant activity (AA) was evaluated by the 2,2 –diphenyl-1-picrylhydrazyl (DPPH) free radical method described by Brand-Williams *et al.* (1995). The DPPH solution (0.1 mM) was prepared by dissolving 3.93 mg of DPPH in 100 mL methanol. Samples of 1 g of freeze-dried peel were immersed in 15 mL of methanol and were sonicated during 20 min in an ultrasonic bath (Cole-Parmer, Pasadena, USA). The supernatant was collected and evaporated until dry. From the extracts, different concentrations were prepared (0.001, 0.01, 0.05, 0.1, 0.5, 0.6 and 0.8 mg mL^{-1}); 1 mL of these extracts solutions and 3 mL DPPH solution were mixed and incubated for 30 min in the dark, at room temperature. Absorbance of the reaction solution (extracts with DPPH solution) was measured at 516 nm with a spectrophotometer (Spectronic 20®, Florida, USA). DPPH radical-scavenging capacity was measured as percentage of inhibited DPPH and was quantified with equation: % inhibited DPPH = **[**$(A_b - A_s) \times 100] / A_b$, where A_b is the absorbance of the control (DPPH 0.1 mM), and A_s is the absorbance obtained from each sample after 30 min with 0.1 mM DPPH. The inhibition percentages calculated with equation were graphed in function of the concentration of the extracts of the treatments (mg mL^{-1}) to obtain the values of median inhibitory concentration of the samples (IC_{50} = mg mL^{-1}), which represented their radical scavenging capacity. IC_{50} is the sample concentration required to trap 50 % of the DPPH free radicals. In this study the radical scavenging capacity of quercetin, one of the most potent antioxidants, was used as reference.

Sensorial determination of suitable sweetness

Preparation of sweetened samples was done similarly as for the freeze drying study previously described (slices were immersed in aqueous solutions of neotame with concentrations of 11, 22, 33, 44 and 55 mg L^{-1} (w/v), 1:1.5 (g:mL) peel:solution, during 1 h). Determination of suitable sweetness was made following the constant stimulus method developed by McBride and Booth (1986). One hundred untrained panelists participated in the sensory evaluation; five samples were randomly presented to each, in codified (with three random digits) number zero plastic cups. Each plastic cup had five xoconostle peel slices (from the same treatment) of 0.1 g each one. Panelists were asked to evaluate one sample at time, and to indicate if they perceived them as “sweet”.

Statistical analysis for suitable sweetness determination

Percentages of panelists which considered each concentration sample as sweet (% of answers “it is sweet”) were transformed into Z values. A linear simple regression was carried out with Z values (dependent variable) vs neotame concentration (independent variable) in order to obtain the slope and intersection of the line. The concentration corresponding to a Z = 0 was calculated with the regression equation; this concentration was considered as the point of subjective equality (PSE). This same regression equation was used to calculate the

just noticeable difference (JND) for the lower level of the normal distribution, calculating the difference between the concentrations for Z = 0 and Z = -0.675 values (McBride and Booth, 1986; Hernández, 2007).

Statistical analysis

Allotment of treatments to experimental units was done with a completely random experimental design. When the analysis of variance (ANOVA) revealed significance, the least significant difference (LSD, $p \leq 0.05$) was used. The experimental unit was made up of 40 g fresh xoconostle tissue, and three replications were evaluated. The response variables evaluated were moisture content, a_w, total phenol content, and antioxidant capacity. The data were analyzed using the SAS software package version 9.1 (SAS, 2006).

RESULTS AND DISCUSSION

Effect of sweetening time on drying by hot air convection

Preliminary evaluations showed that sweetening during 5 h or more decreased flavor, likely because organic acids and other substances responsible for the fruit's flavor were leached from the xoconostle peel. For this reason, the selected sweetening times were in the range between 0 and 3.5 h. None of the sweetening times dehydrated the tissues ($p > 0.05$), and initial moisture content remained between 8.41 and 10.33 g H_2O g^{-1} dry solid and initial aw between 0.991 and 1.000 (Figure 1).

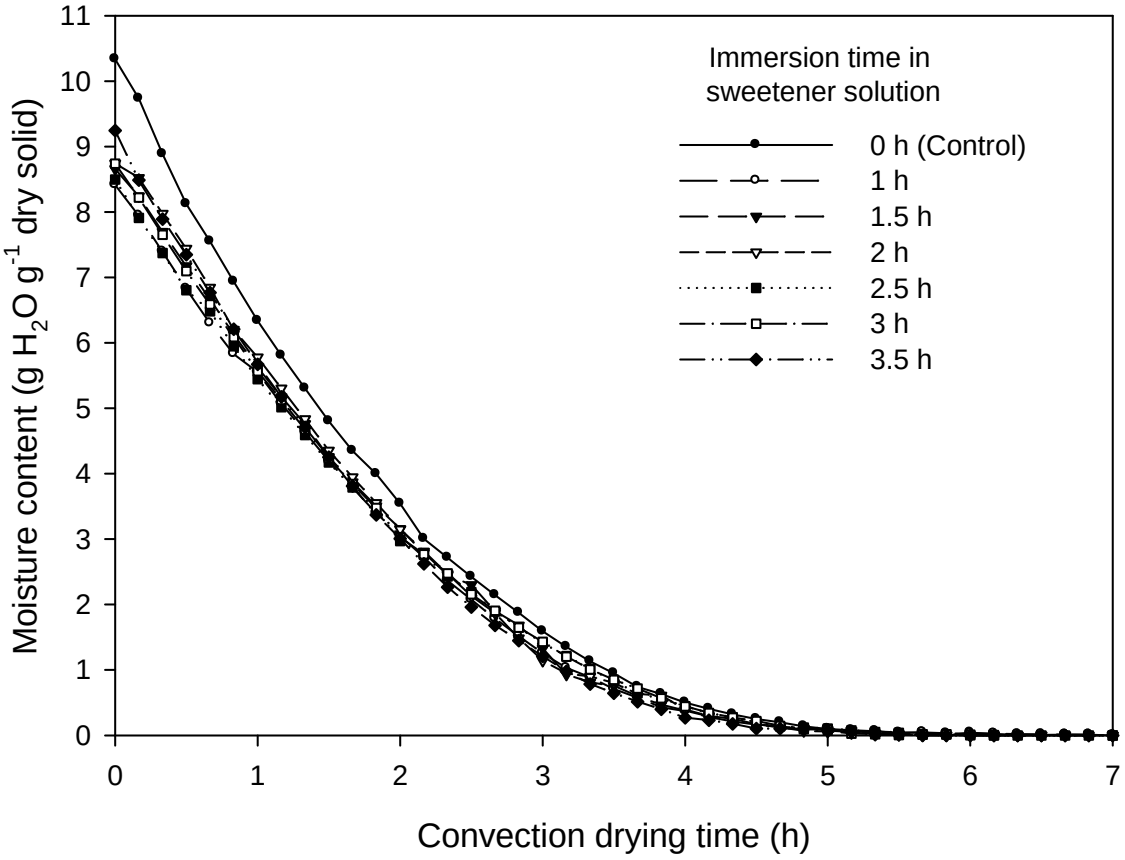

Figure 1. Moisture content (g H_2O g^{-1} dry solid) in function of drying time with convective heat (55 ºC) of xoconostle (*Opuntia matudae*) peel sweetened previously by immersion in aqueous neotame solution (11 mg L^{-1}) during different periods (h).

In the first 2 h the drying rate was high. The slope showed little change between 2 and 3 hours. Then, a gradual reduction in drying rate was observed between 3 and 7 h, similarly to the three typical fruit-drying phases described by Fito *et al*. (2001). According to Hernández and Quinto (2005) the dynamics of convective drying is mainly by diffusion of water. Accelerated loss of water in the first phase may have been because the water was not strongly linked to the solid tissue (Roberti Perez, 2011), since during the drying process, plant tissues first remove free water (Heldman and Singh, 1981). The drying curves in Figure 1 were similar to those obtained by Lahsasni *et al.* (2004) with sweet cactus pear from *O. ficus indica*, which follow the typical food dehydration models, referred by Geankoplis (1998). Xoconostle peel did not completely dehydrate until after 7 h, regardless of sweetening time. An important a_w drop was observed only after 3 h of convective drying (Figure 2). The first

samples which presented a_w values lower than 0.4 were those from the treatments with longer sweetening time (3 and 3.5 h); this occurred little after 4 h of drying. Samples from the treatments with 2, 1.5 and 1 h of immersion in neotame solution reduced its a_w lower than the critical level only after 5 h of drying; the last samples to reach this a_w level were those of the control (after 6 h of drying).

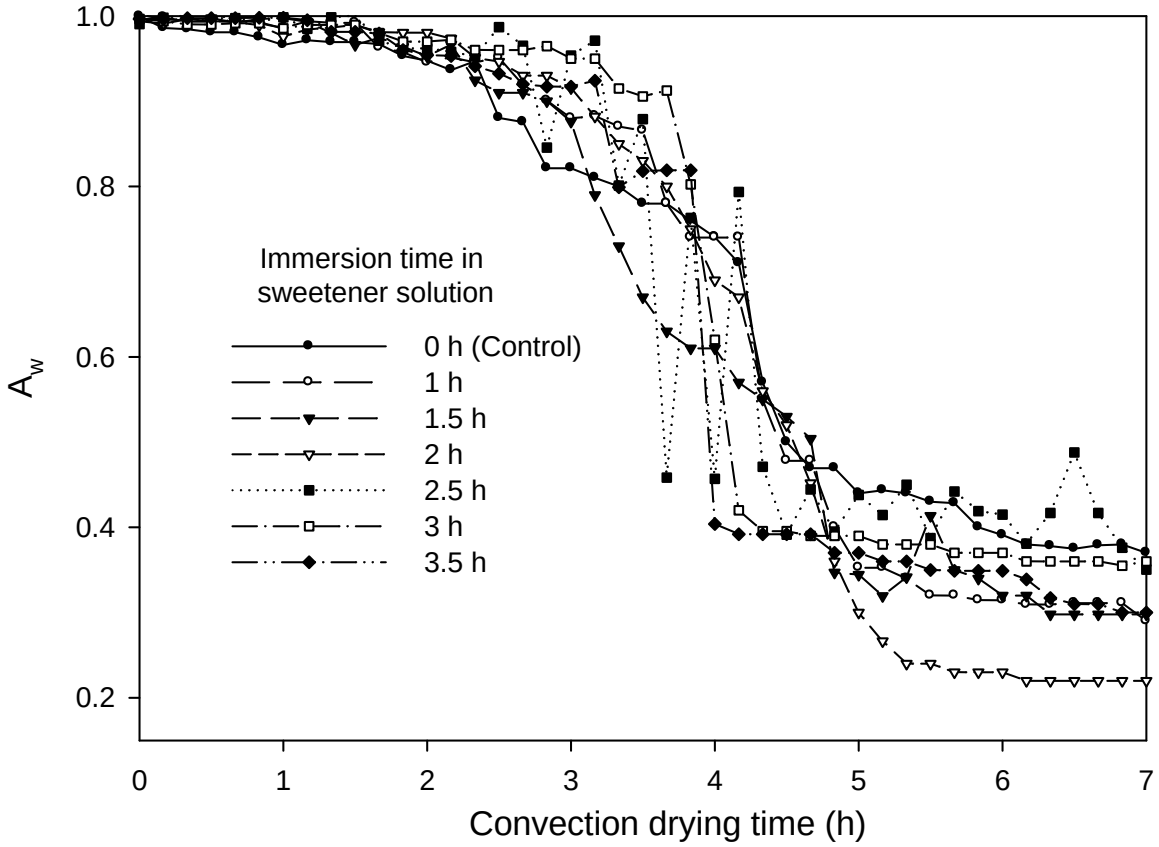

Figure 2. A_w in function of drying time with convective heat (55 ºC) of xoconostle (*Opuntia matudae*) peel sweetened previously by immersion in aqueous neotame solution (11 mg L^{-1}) during different periods (h).

Effect of neotame concentration on freeze-drying

Neotame concentration significantly modified moisture content of the samples; in fact, at the beginning of freeze-drying the initial moisture content fluctuated between 6.0 and 19.62 g H_2O g^{-1} dry solid (Figure 3). In contrast, a_w was not significantly modified by neotame concentration. The initial values of a_w fluctuated between 0.997 ± 0.001 in the treatment without neotame and 0.994 ± 0.001 in the treatment with the highest concentration. In this freeze-drying process the three typical fruit drying phases, described by Fito *et al*. (2001) were not clearly observed. Samples previously sweetened with the highest neotame concentration had the lowest moisture content from the beginning and during the entire

freeze-dehydration process. This may be due to partial dehydration of the samples during neotame treatment. In general, an important a_w drop was observed only after 4 or 5 h of freeze-drying (Figure 4). Concentration of neotame of the previous sweetening affected the dehydration rate or speed. Samples which presented a_w values lower than 0.4 first were those from the treatments with 44 and 33 mg neotame L^{-1}; after 7 h of dehydration these samples showed significantly lower a_w values than the other treatments. By 8 h of freeze-drying, a_w in samples from all treatments with sweetener reached values below 0.4, the value considered suitable for safe storage of dehydrated samples, which prolongs shelf life, and generally reduces oxidation, hydrolytic reactions, enzymatic activities to a minimum, among other effects (Rahman and Sablani, 2009). In contrast, over the same time, a_w of the control remained high (0.85). In fact, control samples reached a_w values lower than 0.4 only after more than 11 h of freeze-drying.

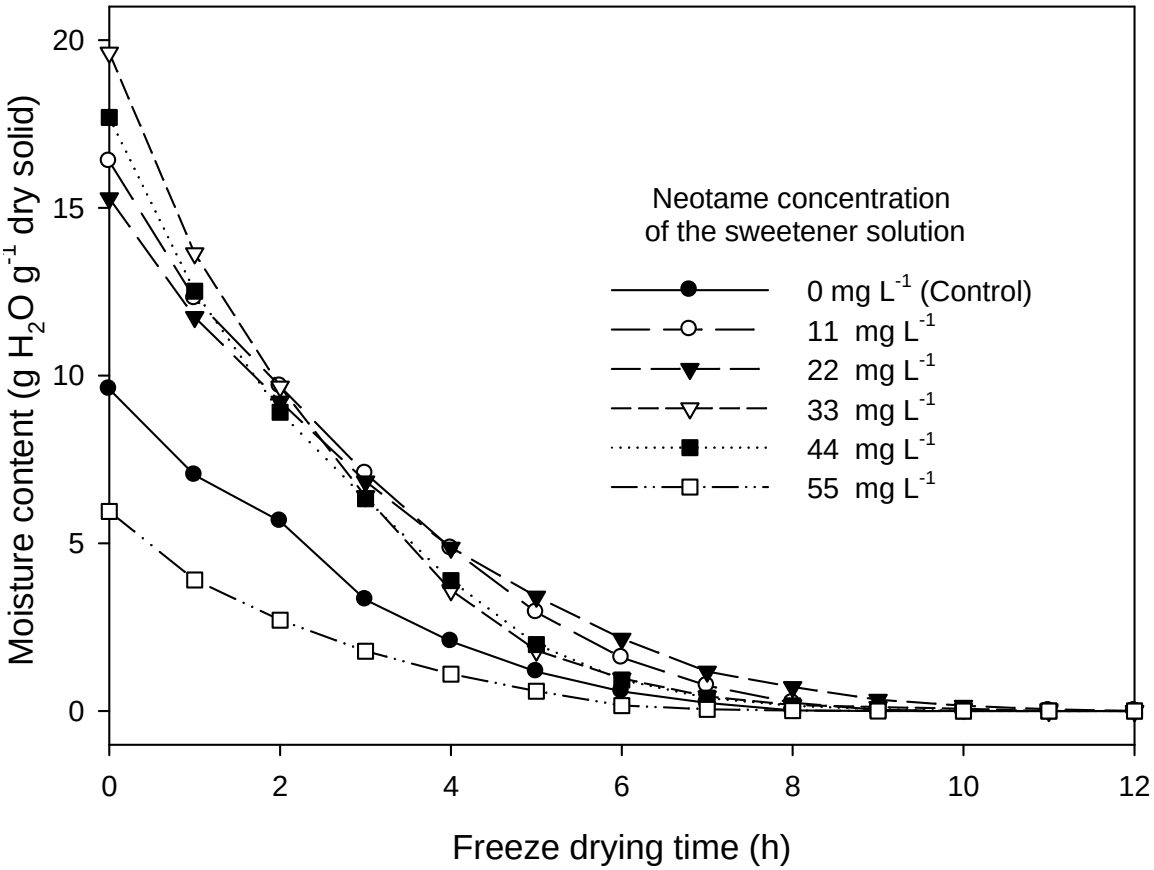

Figure 3. Moisture content (g H_2O g^{-1} dry solid) in function of drying time by freeze-drying (-40°C and 12 Pa) of xoconostle (*Opuntia matudae*) peel sweetened previously with different concentrations of neotame (mg L^{-1}) by immersion in aqueous solution.

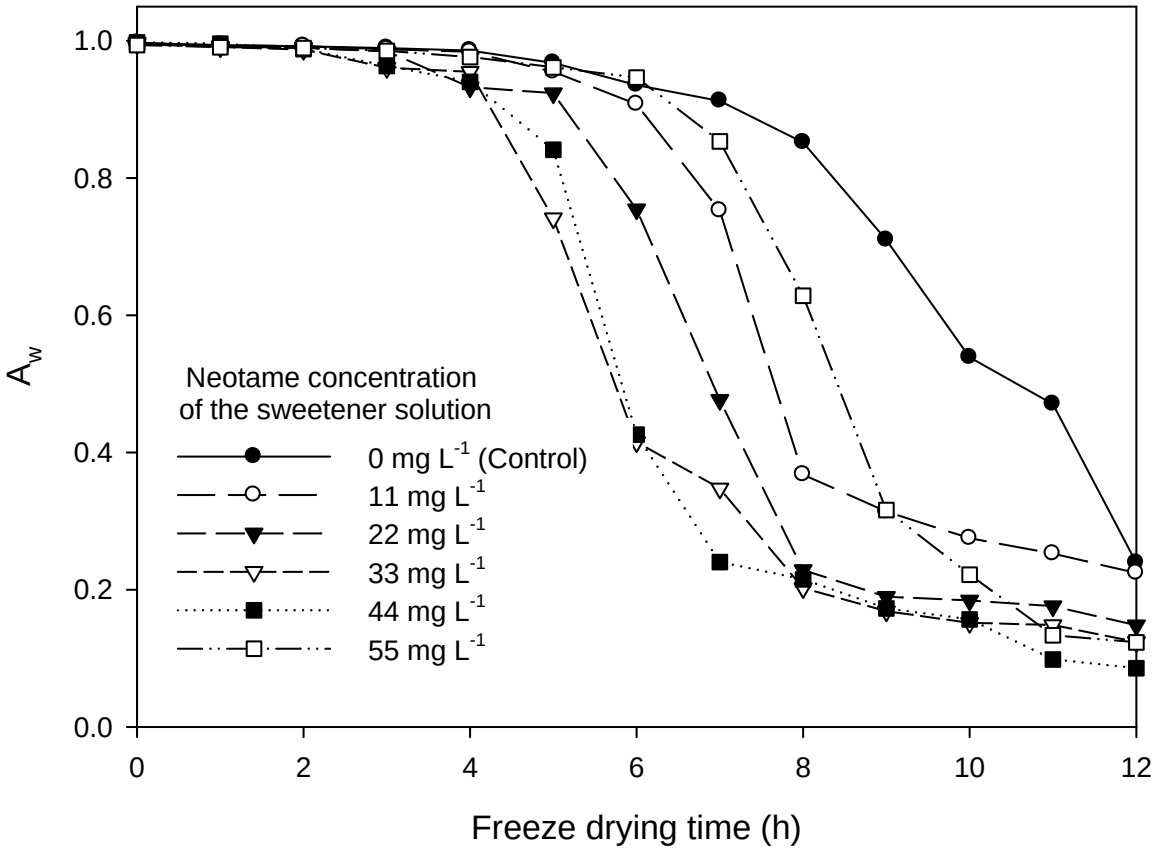

Figure 4. A_w in function of drying time by freeze-drying (-40 °C and 12 Pa) of xoconostle (*Opuntia matudae*) peel sweetened previously with different concentrations of neotame by immersion in aqueous solution.

Total phenolic content in xoconostle sweetened with neotame and dried by freeze-drying

Samples of control showed total phenolic content 13.6 % higher than the average of the sweetened samples from all treatments. This content tended to decrease with increases in sweetener concentration (Table 1). This does not necessarily imply that there was a disappearance of phenolic compounds. In reality the percent reduction of these compounds was caused by the increment of dry weight of the sample by the addition of the sweetener.

Table 1. Total phenolic content and antioxidant activities using DPPH in xoconostle (*Opuntia matudae*) peel sweetened with different concentration of neotame aqueous solution and freeze-dried.

Concentration of neotame (mg L^{-1})	Total phenolic (mg GAE 100 g^{-1})[A]	IC_{50} (mg mL^{-1})[B]
0	1747.50 ± 43.30 a	0.46 ± 0.06 c
11	1572.50 ± 86.60 b,c	0.57 ± 0.08 b
22	1585.00 ± 119.24 b	0.52 ± 0.03 b,c
33	1539.17 ± 31.46 b,c	0.61 ± 0.03 b
44	1535.00 ± 12.50 b,c	0.61 ± 0.11 b
55	1460.00 ± 50.00 c	0.72 ± 0.03 a
CV (%)[C]	4.28	11.94

Arithmetic means of three replications ± standard deviation. Different letters within a column indicate significant statistical differences ($p \leq 0.05$) according to the least significant difference (LSD).

[A] Milligrams of gallic acid equivalents/100 g dry matter.

[B] IC_{50} was determined as the dry matter concentration required to inhibit 50 % DPPH in 30 min.

[C] Coefficient of variation.

The reason for this result could be that the phenolic compounds are linked to aminoacid residues from the neotame peptide by any of several mechanisms in the aqueous medium including hydrogen bonds, covalent bonds, hydrophobic interactions and ionic bonds. The polar rings of the phenolic compound structure have the potential to interact with protein through hydrophobic associations (Rubino *et al.,* 1996; Prigent *et al.,* 2003). In addition, these secondary metabolites may be free or linked in different ways to the compounds present in foods (Jeong *et al.,* 2004; Kumar *et al.,* 2006), which would also explain the decrease in antioxidant activity with increases in sweetener concentration.

It should be noted that the phenolic concentration of the sweetened samples of our study was almost double than that quantified by Guzmán-Maldonado *et al.,* (2010) for the same species, but lower than the concentration reported by Morales *et al.* (2012) for *Opuntia matudae*, cv. Rosa. These differences may be due to distinct cultivation conditions of the plants used in the two studies, since fruits of the genus *Opuntia*, like those of most species, modify their secondary metabolite concentrations in different environments (Figueroa-Cares *et al.,* 2010). Also, the maturity of the fruits evaluated may have been different since the concentration of phenols decreases with ripening in some fruits (Lincoln and Zeiger, 2002). However, it appears that there are no studies that have quantified contents of these phytochemicals during xoconostle fruit ripening. In this study, the phenolic content of xoconostle was lower than that found in other species such as white guava (*Psidium guajava* L.) (Thaipong *et al.,* 2006), orange (*Citrus sinensis*), kiwi (*Actinidia deliciosa*) (Cieslik *et al.,* 2006), strawberry

(*Fragaria vesca*) (Proteggente *et al.,* 2002), and "pitaya" (*Stenocereus stellatus* Riccobono) (Beltrán-Orozco *et al.,* 2009).
Alterations in tissues, such as peeling and slicing, can modify phenol content (De Ancos *et al.,* 2009; Pirovani *et al.,* 2009), which increases in lettuce (*Lactuca sativa*), carrots (*Daucus carota*), celery (*Apium graveolens*), and yams (*Ipomoea batatas*), while it decreases in zucchini (*Cucurbita pepo*), radish (*Raphanus sativus*), and purple cabbage (*Brassica oleracea*) (Cantos *et al.,* 2001; Pirovani *et al.,* 2009; Reyes *et al.,* 2007).

Antioxidant activity in xoconostle peel sweetened and dehydrated by freeze-drying

Preliminary evaluations showed that the radical scavenging capacity of the control was not affected by freeze-drying. In contrast, sweetening before freeze-drying reduced ($p \leq 0.05$) antioxidant capacity, almost in direct proportion to the neotame concentration in sweetener solutions. The trend observed in Table 1 is that the higher concentration of neotame, the higher the IC_{50} values, in other words, increases in solute concentration caused reductions in the xoconostle sweetened slices antioxidant activity. However, although the xoconostle peel without sweetener had 23.7 % more antioxidant capacity than any of the sweetened peel, this antioxidant activity was considered low since a larger quantity of xoconostle sample (0.46 mg mL^{-1}) was required of the reference reactive quercetin (0.000243 mg mL^{-1}) to reduce DPPH $absorbance_{516}$ to 50 % (Table 1).

Antioxidant capacity of fruits and vegetables is associated with phenolic compounds and also with the presence of other metabolites such as vitamin C, carotenoids and some sulfur compounds (Brat *et al.,* 2005). Different authors highlight the fact that contribution of phenolic compounds to antioxidant activity (either favored or reduced) will depend on their type and concentration and by other compounds present in the sample (Villaño *et al.,* 2007; Nsimba *et al.,* 2008; Ozsoy *et al.,* 2009).

Guzmán-Maldonado *et al.* (2010) mention that the xoconostle mesocarp is rich in vitamin C (208.7 mg ascorbic acid 100 g^{-1} DM); the reduction in antioxidant capacity after sweetening could be due to leaching of phenols and vitamin C during the process (De Ancos *et al.,* 2009).

Sensorial determination of adequate sweetness of xoconostle peel sweetened with neotame and dehydrated by freeze-drying

The panelists detected differences in sweetness among the neotame treatments with different concentrations of the sweetener. Thus, the percentage frequency of the response "it is sweet", with the increment in neotame concentration, increased from 6, with the lowest concentration, to 58, with the highest concentration (Table 2). With the response percentages, Z values for each sweetened treatment were obtained (Table 3) and graphed in function of the neotame concentration, and the equation of the line obtained was: $Z = 39.541C - 1.8844$ ($R^2 = 0.9508$). With this equation, it was calculated that 48 mg neotame L^{-1} is the concentration of neotame in xoconostle peel necessary to obtain the PSE value of Z = 0, equivalent to 50 % of the responses "it was sweet" (Gescheider, 1985), and 31 mg neotame L^{-1} for 25 % positive responses. With the difference between these two values, a

JND of 17 was obtained; the Weber JND/PSE proportion, which indicates the proportional increase in the stimulus necessary for a barely detectable difference, was 35.41. Thus, with an increase in neotame concentration of 32 mg L^{-1}, the consumer would not perceive a difference. This determination allows minimizing the investment in neotame, while assuring acceptance of xoconostle sweetness.

Table 2. Frequency of volunteer evaluators responses as affected by the neotame sweetener solution concentration in frozen and then lyophilized xoconostle slices.

Sweetener solution concentration (mg neotame L^{-1})	Number of volunteer evaluators who responded	
	It is sweet	It is not sweet
11	6	94
22	15	85
33	38	62
44	42	58
55	58	42

Table 3. Z values for the probability of different neotame sweetener solution concentrations in frozen and then lyophilized xoconostle slices.

Sweetener solution concentration (mg neotame L^{-1})	Probability	Z Value
11	0.060	-1.555
22	0.150	-1.037
33	0.380	-0.306
44	0.420	-0.202
55	0.580	0.202

CONCLUSIONS

An end product with improved sensorial characteristics was obtained with dehydration by freeze-drying. Sweetening time between 1 and 3.5 h practically did not affect the dynamic of drying by convective hot air. However, sweetening generally reduced the time needed to get a_w reliable values (below to 0.4); maximum reduction (29.16 %) was obtained with 3 or 3.5 h of immersion. The minimum time needed to get an effective sweetening (sensory detectable) was 1 h using a solution with 11 mg neotame L^{-1}.

Concentration of neotame affected the freeze-drying dynamic. Samples with the highest neotame concentration had the lowest moisture content since the beginning and during the entire freeze-dehydration process. Sweetening also generally reduced the time needed to get a_w reliable values (below to 0.4); maximum reduction (45.6 %) was obtained with concentrations of 33 and 44 mg neotame $L^{-1.}$

Sweetening in general affected the percent proportion of TP and AA of the product; both were higher in control samples.

For suitable sweetening, xoconostle slices should be kept in solution of 32 mg neotame L^{-1} for at least 1 h to obtain a physically, chemically, and microbiologically stable (by freeze-drying) and sensory-acceptable product for its pleasant sour-sweet taste.

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