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Sweetened nopal flakes: a functional snack

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Summary

Nopal (*Opuntia* spp. and *Nopalea* spp. genera) is a crop, recognized for its nutritional and medicinal properties; however, there are some underused species, despite the great genetic diversity in Mexico. The genus *Opuntia* spp. is the most consumed nopal, whereas *Nopalea* spp. has low commercial demand, possibly because their nutraceutical attributes are unknown. Additionally, the nopal pads or cladodes are little accepted by many consumers, due to their texture and flavor.

The study objectives were 1) evaluate the nutraceutical content and antioxidant activity of four nopal cultivars: *Nopalea cochenillifera* cv. Texas (NT) and *Opuntia ficus-indica* cv. Jade (OJ), Milpa Alta (OMA), and Atlixco (OA); 2) develop nopal flakes, sweetened with rebaudioside A, from the cultivar with the best nutraceutical quality and sensory acceptability. Ascorbic acid, total phenolics, and total flavonoids were determined by spectrophotometric methods, individual flavonoids (quercetin, kaempferol, and isorhamnetin) by HPLC, and antioxidant activity by the DPPH assay. OA was the cultivar with the best nutraceutical quality.

The sweetened nopal flakes of OA, at a concentration of 1.1 mg g^{-1} rebaudioside A, had the highest sensory acceptability by the panelists in intensity and sweetness preference. The addition of rebaudioside A improved the product's flavor and contributed to preserve the flavonoids and antioxidant activity. These results will contribute to the chemotaxonomy of *O. ficus-indica* and *N. cochellinifera* species, and to the utilization of nopals as functional foods, due to their nutraceutical quality.

Keywords: antioxidant activity, chemotaxonomy, Nopalea, Opuntia, nutraceuticals, rebaudioside A.

Introduction

Nopal is an endemic plant of America that is distributed as wild or cultivated forms in desert and semi-desert areas of the continent. Nopal belongs to the Cactaceae family and includes the *Opuntia* and *Nopalea* genera. *Opuntia* spp. is the largest genus with 377 identified species; 104 of them are found in wild-type form in Mexico (Anaya-Pérez, 2001; Stintzing and Reinhold, 2005). Despite the great diversity of nopal cultivars, few studies have been done to characterize their nutraceutical components, which are metabolites that prevent diseases and maintain the good health of consumers. In the nutraceutical, medicinal, and chemotaxonomical context, the 'Jade' and 'Texas' cultivars from the *Opuntia* genus haven been little studied, whereas these properties are unknown in species from the *Nopalea* genus.

Nopals have been consumed since ancient times for their diverse medicinal properties, such as antidiabetic (Shane-Mcwhorter, 2009), anticholesterolemic (Yang et al., 2008), anticarcinogenic (Abou-Elella and Ali, 2014), anti-inflammatory (Park et al., 2001), antiulcerogenic, diuretic and antioxidant (Alimi et al., 2010) effects. Moreover, nopals have been used for their cicatrizing properties in dermal wounds and gastric ulcers (Alimi et al., 2010), which have been attributed to their content of dietary fiber (polysaccharides), mucilage (polysaccharide), amino acids, vitamins, minerals, and phenolic compounds (Osuna-Martínez et al., 2014).

In recent years, steviol glycosides (chemical compounds identified in *Stevia rebaudiana*, a native plant of South America) have obtained relevance in the food, cosmetic, and pharmaceutical industries, since they are non-caloric sweeteners. Rebaudioside A is the most abundant steviol glycoside in *S. rebaudiana*, the most accepted by consumers, and has no bitter flavor. *In vitro* and *in vivo* studies have reported that rebaudioside A has no mutagenic, teratogenic, or fertility effects (Aranda-González et al., 2014).

The nopal pads or cladodes are little accepted by many consumers, due to its mucilaginous texture, acidity, astringency, and herbal flavor. As an alternative to increase their utilization and consumption, nopal cladodes were transformed into a functional snack by preparation of dehydrated flakes and sweetened with rebaudioside A to improve their texture and flavor, as well as to maintain the nutritional, medicinal, and nutraceutical properties of nopal. In this context, the objectives were to evaluate the ascorbic acid, total phenolic, and flavonoid (quercetin, kaempferol, and isorhamnetin) contents, and the antioxidant activity of four nopal cultivars (*Nopalea cochenillifera* cv. Texas and *Opuntia ficus-indica* cv. Jade, Milpa Alta, and Atlixco), as well as to develop nopal flakes, sweetened with rebaudioside A, from the cultivar with best nutraceutical quality and sensory acceptability.

Materials and methods

Plant material

Small nopal cladodes (20 days of age) of *O. ficus-indica* cv. Jade (OJ), Atlixco (OA) and Milpa Alta (OMA), and *N. cochellinifera* cv. Texas (NT) were harvested free from diseases, physical damages, and morphological alterations in Campo de Experimental La Nopalera of Universidad Autónoma Chapingo. Samples were freeze-dried (model 7670520, Labconco, Kansas, USA) at -50 °C and 0.1 mbar for 18 h. Each experimental unit consisted of three different plants, and each analysis was done with three replicates.

Quantification of ascorbic acid

The quantification of ascorbic acid was done with the spectrophotometric method described by Dürüst et al. (1997), which is based on color reduction of the indicator dichloroindophenol. The results were expressed in mg of ascorbic acid per 100 g dry weight (mg 100 g^{-1} dw).

Total phenolics

Freeze-dried nopal (0.3 g) was pulverized and mixed with 10 mL of 80% (v/v) MeOH aqueous solution. The mixture was homogenized

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by using a vortex and sonicating for 20 min at room temperature (25 ± 2 °C). The extracts were left to rest for 12 h under refrigeration (4 °C) and darkness. Afterwards, the extracts were filtered with Whatman filter paper No. 1 for the quantification of total phenolics, flavonoids, and antioxidant activity. The content of total phenolics was obtained by the Folin-Ciocalteu method (GÜLÇIN et al., 2004). Total phenolics were expressed in mg of gallic acid equivalents per g dw (mg GAE g^{-1} dw).

Total flavonoids

Total flavonoids were determined, based on the spectrophotometric method described by GÜLÇIN et al. (2011), where 10% (w/v) $AlCl_3$ was used as indicator. The results were expressed in mg of quercetin equivalents per g dw (mg QE g^{-1} dw).

Antioxidant activity

Antioxidant activity was measured by the DPPH assay (KUSKOSKI et al., 2005). DPPH (100 μM) in 80% (v/v) methanol was mixed with the sample, and the reaction was incubated for 60 min. The results were expressed in μmol Trolox equivalents per g dw (μmol TE g^{-1} dw). The percentage of inhibited DPPH was determined by using the formula

$$\%DPPH_{inhibited} = (Abs_{blank} - Abs_{sample})/(Abs_{blank})*100 \quad \textbf{(Eq. 1)}$$

where Abs = absorbance and blank = DPPH solution at a concentration of 100 μM.

Identification and quantification of individual flavonoids

Identification and quantification of individual flavonoids was performed, based on the methodology reported by GRAY et al. (2006). Freeze-dried nopal powder (1.5 g) was blended with 25 mL of ethanol and 10 mL of distilled water. The mixture was sonicated for 30 min at room temperature. Thereafter, 4 mL of concentrated HCl were added and the solution was maintained at reflux for 3 h. The volume of the cold mixture was completed to 50 mL with ethanol and was filtered through a Millipore nylon membrane (0.22 μm). The extracts were analyzed with a reverse phase HPLC system (Shimadzu, Kyoto, Japan), equipped with a diode array detector (DAD) and a C18 analytic column (250 × 4.6 mm i.d.: 5 μm) (Phenomenex® 110A, Germany). The injection volume was 10 μL. As mobile phase, a mixture (50:50, v/v) of methanol and an aqueous solution of phosphoric acid (0.5%) was used in isocratic conditions, with a flow rate of 1.2 mL min^{-1}, and a total elution time of 17 min. Column temperature and pressure were 35 °C and 1900-1910 psi, respectively. Quercetin, kaempferol, and isorhamnetin were detected at 252 nm and identified by using their respective standards.

Preparation of sweetened nopal flakes

For the preparation of nopal flakes, homogenous nopal cladodes were used with a weight of 187 ± 5.26 g from the OA cultivar, which presented the highest phenolic content and antioxidant activity, compared with the other cultivars. Fresh, disinfected nopals were cut transversely into flakes (3 mm thick). The flakes were submerged in different solutions of the sweetener rebaudioside A (2, 4, 6, and 8 g L^{-1}) for one hour in a 1:1.5 proportion (nopal:solution). Unsweetened flakes were used as control.

Previously, the optimal freeze-drying time was determined by drying kinetics and desorption isotherms with three different concentrations of rebaudioside A (0, 5, and 10 g L^{-1}) at a temperature of -50 °C and a pressure of 0.01 mbar. Equilibrium moisture content and water activity (a_w) of the flakes were measured in 3-h intervals until concluding 21 h. Equilibrium moisture content was determined with the equation (GEANKOPLIS, 1998)

$$X_t = (m_i - m_{ss})/m_{ss} \quad \textbf{(Eq. 2)}$$

where X_t = equilibrium moisture content (g of water g^{-1} dry weight); $m_{i\,=}$ flake weight (g) per time; m_{ss} = flake dry weight (g). Moisture content was determined according to AOAC method and a_w with a a_w meter (AquaLab, Decagon Devices, Washington, USA).

Afterwards, both sweetened and unsweetened flakes were frozen in liquid nitrogen and then dehydrated by freeze-drying. The freeze-dried flakes were covered with aluminum foil and placed in a desiccator to prevent the absorption of moisture.

Quantification of rebaudioside A

Sweetened and pulverized flakes (2.0 g) were blended with 10 mL of 70% (v/v) ethanol solution. The mixture was placed in a water bath at 70 °C for 30 min. Subsequently, the extracts were filtered with a Millipore nylon membrane (0.22 μm). The extracts were analyzed in a reverse phase HPLC system (Perkin Elmer, USA), equipped with a DAD and a C18 analytic column (150 × 4.6 mm i.d.: 5 um) (Thermo Scientific® Hipersil ODS). Injection volume was 10 μL. As mobile phase, acetonitrile and water (80:20) were used, acidified to pH 3 with phosphoric acid, with a flow rate of 1 mL min^{-1} and a total elution time of 3.5 min. Column temperature was room temperature. Rebaudioside A was detected at 210 nm and identified by using its respective standard.

Sensory evaluation

Sensory evaluation of unsweetened flakes and flakes sweetened with different concentrations of rebaudioside A (2, 4, 6, and 8 g L^{-1}) was done to identify the rebaudioside A concentration with most acceptability by the panelists. The evaluation was done by applying two reference scales: intensity (just about right, JAR) and preference (hedonic). The panel was made of 100 non-trained judges (53 men and 47 women) of 15-26 years of age. Each panelist evaluated at random the flakes with the five different concentrations of rebaudioside A.

Statistical analysis

The data were analyzed using a randomized complete block design (RCBD), and statistical differences were determined by an analysis of variance (ANOVA), followed by Tukey's mean difference test ($p < 0.05$). All data were analyzed by using SAS software.

Results and discussion

Nutraceutical content and antioxidant activity in four nopal cultivars

The contents of ascorbic acid, total phenolics, total flavonoids, antioxidant activity, and inhibited DPPH after 60 min of reaction are presented in Tab. 1 for the cultivars OJ, OA, and OMA (*O. ficus-indica*) and NT (*N. cochellinifera*). The ascorbic acid content in the OMA and OA cultivars was superior (38.5 and 43.1 mg 100 g^{-1} dw, respectively) to the same cultivars (19.21 and 25.52 mg 100 g^{-1} dw, respectively) reported by RAMÍREZ-MORENO et al. (2013); but MEDINA-TORRES et al. (2011) found a greater concentration (205.62 mg 100 g^{-1}) for the OMA cultivar recollected from different regions to that of the present study. On the other hand, the concentration of phenolic compounds in the studied cultivars (2.9-8.1 mg GAE g^{-1} dw) was within the interval published by GUEVARA-FIGUEROA et al. (2010) for other Mexican cultivars of the *Opuntia* genus: Tapón (I and II), Blanco, and Manso (2.0, 5.2, and 11.7 mg GAE g^{-1} dw, respectively). Total flavonoids are not affected by mechanical damages or manipulation of the food, just as it has been reported in other

Tab. 1: Nutraceutical content and antioxidant activity of four nopal cultivars.

Cultivar	Ascorbic acid (mg 100 g^{-1} dw)	Total phenolic compounds (mg GAE g^{-1} dw)	Total flavonoids (mg QE g^{-1} dw)	Antioxidant activity (µmol TE g^{-1} dw)	DPPH Inhibited (%)
NT	28.4 ± 1.51 d	4.0 ± 0.51 b	1.9 ± 0.01 bc	5.9 ± 0.58 b	42.6 ± 4.06 b
OJ	48.9 ± 1.11 a	2.9 ± 0.27 c	2.0 ± 0.14 b	8.1 ± 0.16 a	57.2 ± 1.11 a
OA	43.1 ± 0.42 b	8.1 ± 0.46 a	1.7 ± 0.06 c	8.3 ± 0.35 a	58.4 ± 1.18 a
OMA	38.5 ± 1.51 c	4.6 ± 0.21 b	2.3 ± 0.04 a	8.2 ± 0.17 a	58.9 ± 2.40 a

Values represent the mean ± standard deviation of 3 replicates. Different letters in the same column indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: NT = *Nopalea cochellinifera* cv. Texas; OJ, OA, and OMA = *O. ficus-indica* cv. Jade, Atlixco, and Milpa Alta, respectively; GAE = gallic acid equivalents; QE = quercetin equivalents; TE = Trolox equivalents; DPPH = free radical 2,2-diphenyl-1-picrylhydrazyl.

phenolic compounds that respond to stress applied to food, such as storage time, refrigeration temperature, and UV light, conditions which cause an increase in the phenolic content as a defense mechanism (De Ancos et al., 2009). Finally, the cultivars OJ, OA, and OMA presented an antioxidant activity that was superior to cultivar NT (*N. cochellinifera*). The OA cultivar excelled for its high content of phenolic compounds and antioxidant activity, reason for which it was selected to prepare the sweetened flakes.

In Fig. 1 are shown the chromatograms of the flavonoid profile of the four cultivars evaluated in this study. Quercetin, kaempferol, and isorhamnetin were identified in all cultivars. These three flavonoids have been found in several species of the *Opuntia* genus (*O. ficus-indica, O. humifusa, O. monacantha, O. lindheimeri, O. robusta, O. streptacantha, O. undulata, O. rastrera y O. leucotricha)* (Medina-Torres et al., 2011; Jun et al., 2013; Valente et al., 2007; Santos-Zea et al., 2011). Furthermore, glycosylated flavonoids have also been identified in the *Opuntia* genus (quercetin-3-*O*-β-glucopyranoside, quercetin-3-*O*-rutinoside or rutin, kaempferol-3-*O*-rutinoside or nicotiflorin, isorhamnetin-3-*O*-rutinoside or narcissin and isorhamnetin-3-*O*-glucoside) (Guevara-Figueroa et al., 2010). However, these metabolites have not been studied in the *Nopalea* genus. The results of the present study could contribute to the chemotaxonomic differences between the species *O. ficus-indica* and *N. cochellinifera*. In addition, the identification of these flavonoids could explain some of the medicinal properties of nopal.

Fig. 2 depicts the concentrations of quercetin, kaempferol, and isorhamnetin of the cultivars of this study. Isorhamnetin was the flavonoid that was found in the highest concentration in OMA cultivar (3.902 mg g^{-1} dw), whereas the most prevalent flavonoid in the

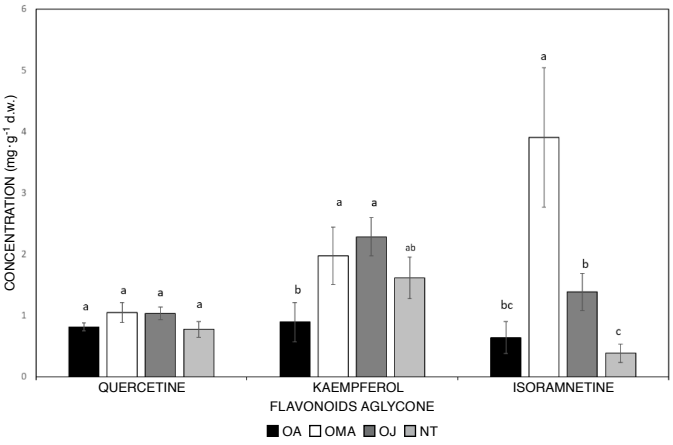

Fig. 2: Flavonoid content in four nopal cultivars. Values represent the mean ± standard deviation of 3 replicates. Bars with different letters for the same flavonoid aglycone indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: dw = dry weight; NT = *N. cochellinifera* cv. Texas; OJ, OA, and OMA = *O. ficus-indica* cv. Jade, Atlixco, and Milpa Alta, respectively.

OJ, OA, and NT cultivars was kaempferol with similar concentrations between them (1.611-2.284 mg g^{-1} dw). The concentrations of the three flavonoids of all cultivars were inferior to those found by Medina-Torres et al. (2011) for the same nopal species (*O. ficus-indica*) (1.995, 2.201, and 4.065 mg g^{-1} dw, respectively). However, the concentrations of kaempferol and isorhamnetin were superior

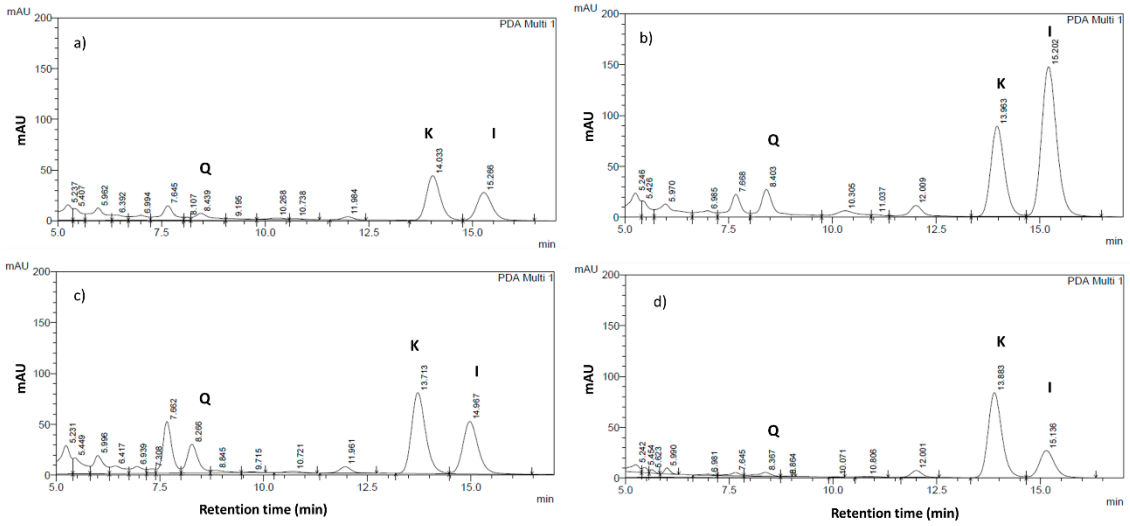

Fig. 1: Chromatograms obtained by reverse phase HPLC of the flavonoid profile identified in four nopal cultivars. **a)** *O. ficus-indica* cv. Atlixco; **b)** *O. ficus-indica* cv. Milpa Alta; **c)** *O. ficus-indica* cv. Jade; **d)** *N. cochellinifera* cv. Texas. Abbreviations: Q = quercetin, K = kaempferol, I = isorhamnetin.

to those reported for the cultivars Jalapa and Villanueva (*O. ficus-indica*) (0.119 and 0.474 mg g^{-1} dw kaempferol, and 0.726 and 0.654 mg g^{-1} dw isorhamnetin, respectively) by SANTOS-ZEA et al. (2011).

The significant differences observed in the nutraceutical content and antioxidant activity between the cultivars of the present study and the differences found with other studies could be due, as in other foods, to diverse factors: genetic (RAMÍREZ-TOBÍAS et al., 2012), physiological response to manipulation (DE ANCOS et al., 2009), ripening stage (BAKHSHI and ARAKAWA, 2006), agronomic practices, environmental conditions (HAGEN et al., 2007), and postharvest handling (TSAO, 2007). In the case of environmental conditions and postharvest handling, plants synthesize flavonoids and other phenolic compounds as a defense mechanism against UV light, water stress, and predators. However, the chemical composition of a food can also affects the content of some metabolites. VILLAÑO et al. (2007) found that the content of phenolic compounds can vary, depending on the composition of the food matrix. In this context, nopal is characterized by a high concentration of mucilage (polysaccharide consisting of galactose, rhamnose, arabinose, and xylose), and its binding to phenolics could explain the low phenolic concentration when only free phenolics are quantified, which could also explain a possible decrease in antioxidant activity (KUMAR et al., 2006).

Nopal flakes from *O. ficus-indica* cv. Atlixco (OA)

Drying kinetics and desorption isotherms

For the preparation of nopal flakes, cladodes from the OA cultivar were used, which presented the highest content of phenolic compounds and antioxidant activity compared with the remaining cultivars. The preliminary study of drying kinetics and desorption isotherms, obtained with different rebaudioside A concentrations (0, 5, and 10 g L^{-1}), allowed to determine the optimal drying time, where moisture content and a_w of the nopal flakes were maintained constant (Fig. 3).

Nopal flakes from OA cultivar, with an initial moisture content of 94.5 ± 0.56% (17.18 g_{H2O} g^{-1} dw) and a_w of 0.987 ± 0.059, were dehydrated until reaching the equilibrium moisture content of 3.36 ± 0.25 % (0.592 g_{H2O} g^{-1} dw) and a_w of 0.217 ± 0.003 at -50 °C and a pressure of 0.01 mbar (Fig. 3a and 3b). From 18 h of freeze-drying onwards, the moisture content and a_w of the nopal flakes were maintained constant; therefore, 18 h was chosen as the optimal drying time for the preparation of nopal flakes, sweetened with rebaudioside A at the concentrations of 0, 2, 4, 6, and 8 g L^{-1}. The concentration of rebaudioside A (0, 5, and 10 g L^{-1}) added to the nopal flakes did not cause a significant effect over the drying kinetics by freeze-drying. However, the flakes sweetened with rebaudioside A had a lower a_w than unsweetened flakes (control) (Fig. 3b). This could have been due to an interaction between rebaudioside A and the flakes' surface, leading to a decrease of available water of the food (SHIVHARE et al., 2004). The desorption isotherms (Fig. 3b) correspond to Type III, according to Brunauer's classification (MATHLOUTHI and ROGE, 2003), characteristic of foods with high content of polysaccharides, such as mucilage of nopal. After 18 h of dehydration, the nopal flakes had a crispy texture and were stable, and the low a_w value is associated with a high level of preservation and shelf-life, since foods with an a_w below 0.4 are resistant to microbial deterioration and are less susceptible to enzymatic reactions (IGATHINATHANE et al., 2007).

Sensory evaluation

Flakes sweetened with 6 g L^{-1} of rebaudioside A were the best evaluated sensorially (Tab. 2), since most panelists (64%) rated their sweet-

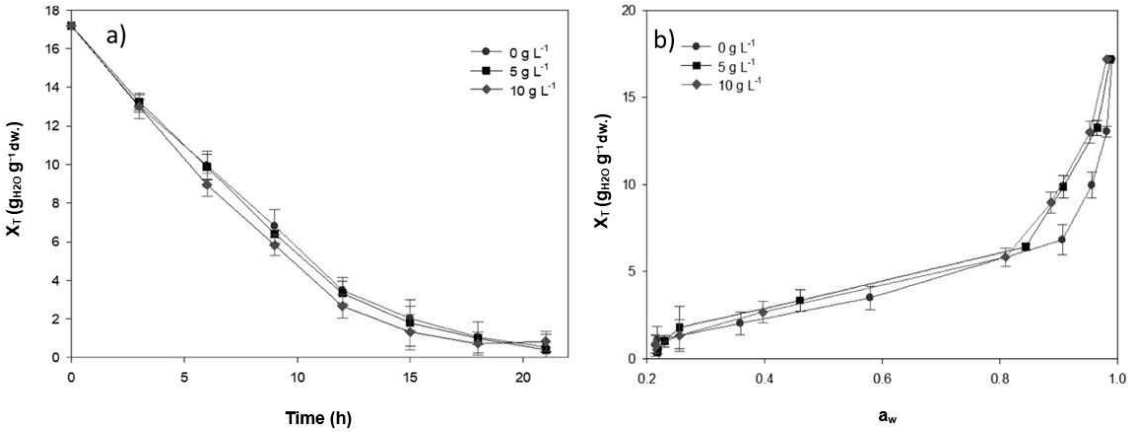

Fig. 3: Drying kinetics (a) and desorption isotherms (b) of nopal flakes of *O. ficus-indica* cv. Atlixco, preliminarily sweetened with three concentrations of rebaudioside A (0, 5, and 10 g L^{-1}). Values represent the mean ± standard deviation of 3 replicates. Abbreviations: X_t = equilibrium moisture content, a_w = water activity, dw = dry weight.

Tab. 2: Sensory evaluation of nopal flakes of *O. ficus-indica* cv. Atlixco, sweetened with different concentrations of rebaudioside A.

Rebaudioside A	JAR scale				Hedonic scale
(g L^{-1})	Response "little sweet" %	Response "adequate" %	Response "very sweet" %	Intensity score	Preference score
0	95	5	0	1.1 e	3.3 c
2	46	48	6	2.2 d	5.3 b
4	31	55	14	2.7 c	5.4 ab
6	12	64	24	3.2 b	5.9 a
8	5	52	43	3.8 a	5.7 ab

Values represent the mean ± standard deviation of 100 panelists. Different letters in the same column indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: JAR = just about right.

ness intensity as "adequate" (JAR scale) and were the flakes that obtained the highest score in preference (hedonic scale) (ROTHMAN and PARKER, 2009). Therefore, these flakes were selected for further analyses.

Incorporated rebaudioside A

HPLC analysis allowed to identify and establish for all treatments the real concentration of rebaudioside A incorporated by immersion to the nopal flakes. Fig. 4 shows the chromatograms of rebaudioside A as a standard (Fig. 4a) and in sweetened nopal flakes (Fig. 4b). The flakes that were best scored in the sensory evaluation were those at a concentration of 6 g L^{-1} of rebaudioside A and presented a concentration determined by HPLC of 1.1 ± 0.013mg g^{-1} of the sweetener (Tab. 3).

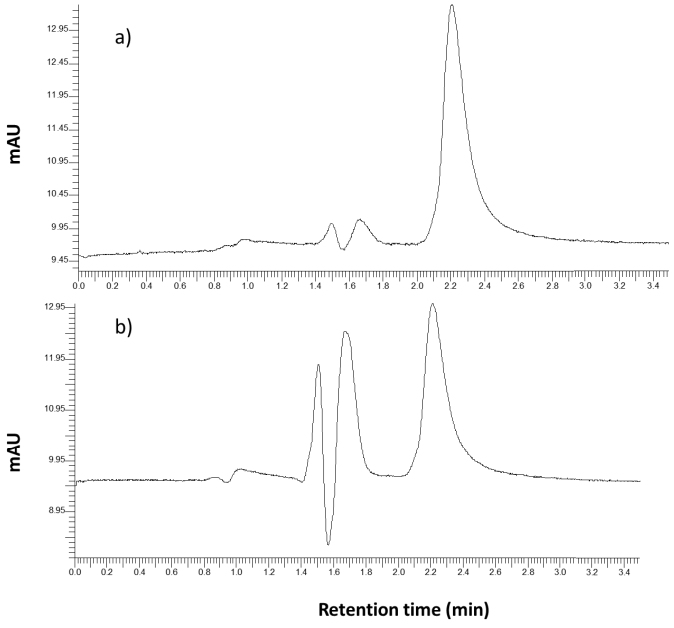

Fig. 4: Chromatograms of pure rebaudioside A (a) and nopal flakes of *O. ficus-indica* cv. Atlixco, sweetened by immersion in a rebaudioside A solution (6 g L^{-1}) (b).

Tab. 3: Concentration of rebaudioside A incorporated to nopal flakes of *O. ficus-indica* cv. Atlixco after being submerged in different solutions of rebaudioside A.

Concentration of rebaudioside A in the immersion solution (g L^{-1})	**Real concentration of rebaudioside A in the nopal flakes** (mg g^{-1})
0	ND
2	0.8 ± 0.04 d
4	1.0 ± 0.05 c
6	1.1 ± 0.01 b
8	1.3 ± 0.03 a

Values represent the mean ± standard deviation of 3 replicates. Different letters in the same column indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: ND = not detected.

Nutraceutical content

The nutraceutical content of the most sensorially accepted sweetened flakes of the OA cultivar (6 g L^{-1}) was evaluated and compared with unsweetened flakes. Tab. 4 shows the levels of ascorbic acid, total phenolics, total flavonoids, and antioxidant activity of both types of flakes. The significant differences found between the nutraceutical content of sweetened and unsweetened flakes could be attributed to leaching of soluble nutraceuticals during immersion in the rebaudioside A solution (NAIDU, 2003). However, KUMAR et al. (2006) explained that a decrease in phenolic compounds could be also due to the presence of hydrophilic interactions with components of the food matrix; in this case, an interaction with rebaudioside A. But, despite the loss of phenolic compounds, the antioxidant activity was less affected, probably caused by rebaudioside A (TAVARI et al., 2015). This phenomenon was observed by CARBONELL-CAPELLA et al. (2013) in papaya, mango, and orange juices, processed with high pressures, where the incorporation of glycosides from *S. rebaudiana* increased the antioxidant activity of these beverages.

Fig. 5 depicts the concentrations of quercetin, kaempferol, and isorhamnetin in sweetened and unsweetened flakes. No significant differences were observed between the flakes for none of the flavonoids, but the amounts were inferior to those published by MEDINA-TORRES et al. (2011) in nopals of *O. ficus-indica* (1.99, 2.20, and 4.06 mg g^{-1}, respectively). However, the authors reported that the content of these metabolites was reduced almost entirely after exposure of convective drying at a temperature of 65 °C.

The consumption of quercetin (2 mg per kg body weight in humans) has shown significant effects in the reduction of triglycerides, choles-

Tab. 4: Nutraceutical content and antioxidant activity of unsweetened flakes and flakes sweetened with rebaudioside A (6 g L^{-1}) of nopal *O. ficus-indica* cv. Atlixco.

Nutraceutical property	Unsweetened flakes	Sweetened flakes	Recovery (%)
AA (mg 100 g^{-1})	38.0 ± 2.21 a	33.9 ± 1.25 b	89.20
TP (mg GAE g^{-1})	5.9 ± 0.05 a	5.0 ± 0.19 b	84.87
TF (mg QE g^{-1})	2.9 ± 0.22 a	2.9 ± 0.11 a	99.74
AOX (μmol TE g^{-1})	9.8 ± 0.09 a	9.0 ± 0.07 b	91.35
(% $DPPH_{inhibited}$)	55.8 ± 0.47 a	51.1 ± 0.39 b	91.71

Values represent the mean ± standard deviation of 3 replicates. Different letters in the same row indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: AA = ascorbic acid, TP = total phenolics, TF = total flavonoids, AOX = antioxidant activity, GAE = gallic acid equivalents, QE = quercetin equivalents, TE = Trolox equivalents, DPPH = free radical 2,2-diphenyl-1-picrylhydrazyl.

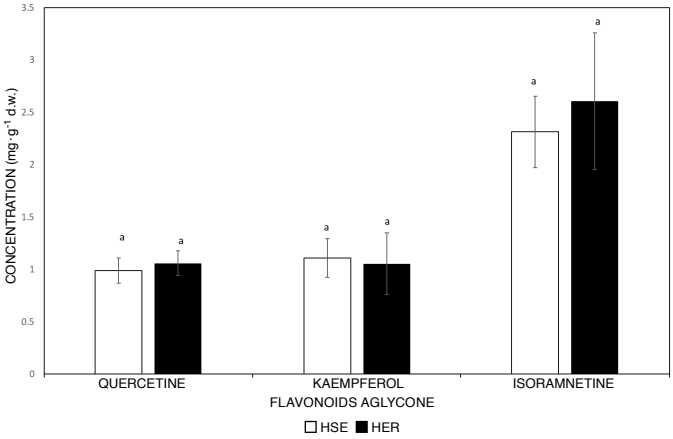

Fig. 5: Concentration of quercetin, kaempferol, and isorhamnetin in unsweetened flakes and flakes sweetened with rebaudioside A (6 g L^{-1}) of nopal *O. ficus-indica* cv. Atlixco. Values represent the mean ± standard deviation of 3 replicates. Bars with different letters for the same flavonoid aglycone indicate statistically significant difference by Tukey's test ($p < 0.05$). Abbreviations: dw = dry weight.

terol, and glucose in blood, as well as decrease in weight gain (Rivera et al., 2008). This dose would be equivalent to eating approximately 130 g of sweetened flakes. Furthermore, it has been reported that kaempferol inhibits proliferation of cancer cells and preserves the viability of normal cells (Chen and Chen, 2013). Moreover, recent studies have revealed that isorhamnetin glucosides of *O. ficus-indica* prevent the development of metabolic abnormalities associated with obesity induced by a high-fat diet and cause anti-inflammatory effects (Antunes-Ricardo et al., 2015) in mice. Finally, steviosides (rebaudioside A and stevioside) provide some benefits to health, such as reduction of blood pressure (Hsieh et al., 2003) and can be consumed by patients with diabetes type I and type II without presenting adverse effects (Barriocanal et al., 2008). Hence, sweetened nopal flakes could be a functional snack and could possibly be consumed by patients with diabetes.

Conclusions

The nopal cultivars with greater commercial demand *O. ficus-indica* presented higher antioxidant activity than the underused NT cultivar from *N. cochellinifera*. The profile of quantified and identified flavonoids in this study could contribute to the chemotaxonomy of both nopal species. The nopal flakes of the OA cultivar (*O. ficus-indica*), sweetened with rebaudioside A (1.118 mg g^{-1}), had a good sensory acceptability. The nutraceutical content of the flakes was decreased during immersion in the rebaudioside A solution; however, the antioxidant activity was preserved, possibly due to the presence of rebaudioside A. Due to their nutraceutical content and antioxidant activity, the freeze-dried and sweetened nopal flakes could be used as a functional snack and could be possibly be consumed by patients with diabetes. The results of this study could contribute to the utilization and consumption of nopal.

Conflict of interest

No potential conflict of interest was reported by the authors.

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