

## Preservation of fresh edible cactus stems (*Opuntia ficus indica* Mill.) by modified atmosphere packaging

Conservación de nopal fresco desespinado (*Opuntia ficus indica* Mill.) en envases con una atmósfera modificada

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**Abstract.** Cactus stems, the cladodes of *Opuntia* spp. cacti, are consumed in Mexico and other countries due to their fresh and herbaceous flavor, and because of their widely known nutraceutical benefits. In order to extend the postharvest life of this vegetable, the effect of a modified atmosphere packaging (MAP) was studied in cactus stems of the cultivar Atlixco stored at  $4 \pm 1$  °C for 20 days under three types of atmospheres: (1) air (passive atmosphere), (2) 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub>, and (3) N<sub>2</sub>. During storage, the titratable acidity decreased and the color of cladodes became darker and less green; however, the 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> atmosphere was able to preserve both quality characteristics. All modified atmospheres reduced weight loss (from 8 to <2%) and the symptoms of chilling injury, and this physiological disorder appeared earlier in controls than in MAP-stored cladodes. The levels of fermentation metabolites were low in all three evaluated atmospheres. Because of this, only cladodes stored under the N<sub>2</sub> atmosphere were selected for further-sensory analysis of the MAP effect on odor perception as evaluated by a trained panel. Results indicated that there was no detrimental effect (atypical odors) of MAP on this sensory characteristic. We conclude that cultivar Atlixco is suitable for preservation using MAP technology.

**Keywords:** Acetaldehyde; Cladodes; Nopal; Ethanol; Modified atmosphere; Quality; Refrigeration; Wound.

**Resumen.** Los nopales o cladodios de las plantas cactáceas de la especie *Opuntia* spp. se consumen en México y otros países debido a su sabor fresco y herbáceo, y a sus bien conocidas propiedades nutraceuticas. Para poder extender la vida poscosecha de esta hortaliza, se estudió el efecto del envasado en atmósfera modificada (EAM) en nopales del cultivar Atlixco almacenado a  $4 \pm 1$  °C por 20 días en tres tipos de atmósfera: (1) aire (atmósfera pasiva), (2) 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> y (3) N<sub>2</sub>. Durante el almacenamiento, la acidez disminuyó y el color de los cladodios se volvió más oscuro y menos verde; sin embargo, la atmósfera de 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> logró preservar ambas características de calidad. Todas las atmósferas redujeron la pérdida de peso (de 8 a <2%) y los síntomas de daño por frío; además, esta fisiopatía apareció primero en los cladodios control que en los almacenados en EAM. Los niveles de metabolitos de fermentación fueron bajos en las tres atmósferas evaluadas. Debido a esto, sólo los cladodios almacenados en una de ellas (la que contenía N<sub>2</sub>) fueron expuestos posteriormente a un análisis sensorial para determinar el efecto del EAM en el olor evaluado por un panel entrenado. Los resultados indicaron que no hubo un efecto negativo (olores atípicos) del EAM en esta característica sensorial. Se concluyó que el cultivar Atlixco puede preservarse aplicando la tecnología de EAM por un periodo de 15-20 días a  $4 \pm 1$  °C.

**Palabras clave:** Acetaldehído; Cladodios; Nopal; Etanol; Atmósferas modificadas; Calidad; Refrigeración; Herida.

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## INTRODUCTION

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In Mexico, the total annual production volume of cactus stems, also called cladodes or nopales (singular, nopal), is of 777413 tons (SIAP, 2012), with crops occupying an area of 12 thousand hectares spread across 25 states in the country. The most important cactus stem cultivars or commercial varieties in terms of total cultivated surface area are Milpa Alta or Esmeralda, Atlixco or Villanueva, Copena V1 and V8, and Nopalea.

In many regions of Mexico, the consumption of cactus stems is deeply rooted in people's diets because of their generally low cost, palatable sour taste, herbaceous aromatic notes, and nutritional and functional characteristics. These include hypoglycemic and anti-diabetic properties (López et al., 2008), as well as ulcer-fighting, anti-hyperlipidemia and cholesterol-reducing effects (Stintzing & Carle, 2005). In light of these nutraceutical benefits and given their content of functional substances (Stintzing & Carle, 2005; Ventura-Aguilar et al., 2013), cactus stems have captured the attention of researchers in various parts of the world, and their demand is expected to increase in the future.

Mexican communities across the United States and Canada searching for ethnic products also generate an important demand. At the same time, the demand for cactus stems in both domestic and export markets could potentially increase in the short term if the product offered adheres to consumer needs and quality expectations. For example, removing the spines and storing the cladode stems in containers of an appropriate size adds an aggregated value that can satisfy the demand of such markets. The processing and packaging of stems in this manner could also contribute to lowering postharvest losses, which are around 15 million Mexican pesos per year due to the fall of prices (to unprofitable ranges) from May to October due to the high offer of the product during this period (Callejas-Juárez et al., 2009).

Scientific reports about the postharvest preservation of whole cladode stems are limited (Cantwell et al., 1992; Cantwell, 1995; Rodríguez-Félix & Villegas-Ochoa, 1997) and virtually non-existent when it comes to stems whose spines have been removed by minimal processing. Temperatures of 4 to 5 °C are typically used to preserve quality attributes and prevent the proliferation of harmful microorganisms (Kitinoja & Gorny, 1999) on minimal-processed products. In fresh (unprocessed) products, adequate temperatures depend on the specific sensitivity to chilling injury (CI), a physiological disorder that occurs when products are stored at sub-optimal temperatures. For unprocessed cactus stems, 5 to 10 °C and a 90-95% relative humidity are recommended (Cantwell, 2007); however, even under these conditions the stems can show CI depending on the time of storage (Rodríguez-Félix & Villegas-Ochoa, 1997). There are no reports dealing with this physiological disorder in cactus stems of the cultivar Atlixco.

Modified atmosphere packaging (MAP), a technique that complements refrigeration, can limit CI in refrigerated products as well as many deteriorative changes associated with senescence (Kader, 1986; Kader, 2002). It commonly involves the use of any one of four different types of atmospheres inside product containers: (1) N<sub>2</sub>, (2) high levels of CO<sub>2</sub>, (3) a mixture of these two gases together with low levels of O<sub>2</sub> (active atmospheres) or (4) atmospheric air of standard composition (passive atmosphere). A disadvantage of preserving produce inside MAP is that the production of fermentation metabolites (acetaldehyde and ethanol) can increase due to the conditions of stress created by the low levels of O<sub>2</sub> and high levels of CO<sub>2</sub> inside the containers (Dangyang et al., 1994; Purvis, 1997; Tadege et al., 1999; Kursteiner et al., 2003; Kato-Noguchi & Yasuda, 2007). However, the production of these metabolites depends on the specific variety or cultivar examined (Watkins et al., 1999; Pelayo et al., 2003; and Ponce-Valadez & Watkins, 2008). As for the effects of MAP on sensory characteristics, increased levels of acetaldehyde and ethanol can have a negative impact on odor and flavor perception if (1) the concentrations of these metabolites overcome their threshold values of sensory perception (Ke et al., 1991), or (2) their increased quantities alter the original proportion of odoriferous compounds (odor profile). There are no published reports examining the sensory odor profiles of fresh unstored cactus stems, nor does information exist concerning the effect that MAP may exert on these profiles.

Therefore, the objective of this work was to evaluate the quality attributes, fermentation metabolites and sensory odor profiles of MAP-stored, spine-free, cactus stems of the cultivar Atlixco in order to test the effectiveness of this preservation technique in maintaining the quality and prolonging their post-harvest life. To our knowledge, there have been no other scientific reports that explore the preservation of this cultivar using MAP.

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## MATERIALS AND METHODS

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**Biological materials.** Organically-grown cactus stems of the cultivar Atlixco (*Opuntia ficus indica*) were collected during the fall from commercial plantations located in Otumba, Estado de Mexico (19° 41' 52.5" N; 98° 45' 25.9" W). These orchards use plastic tunnels to protect the harvest from winter temperatures, organic manure from compost as fertilizer, and natural products for pest control (chili and garlic extracts and biological control agents). Since cactus stems are Crassulacean Acid Metabolism (CAM) plants that fix CO<sub>2</sub> as malic acid at night before converting it into sugars during the day (Rodríguez-Félix & Villegas-Ochoa, 1998; Corrales-García & Flores-Valdez, 2003; Taiz & Zeiger, 2006), the cladodes were harvested early in the morning (6-7 am) to limit changes in acidity. The hand-harvested cladodes were then placed in washed and disinfected plastic grids and transported to the laboratory in an isothermal vehicle.

**Treatment application and experimental design.** Cladodes were selected by size (20 to 25 cm long), uniformity of color, and lack of visible surface defects according to quality norms established by CODEX STAN 185 (1993). They were then disinfected in a 200 ppm solution of active chlorine, and subjected to minimal processing by having their spines removed. This consisted of lightly scraping both cladode surfaces with a knife and cutting around the edges. Processed cactus stems were then placed inside PolySweat bags (Bolco®, D.F., Mexico) made of a 0.035 mm-thick polyethylene monolayer film with the following gas permeation rates: 260.6 g O<sub>2</sub>/100 in<sup>2</sup>/day/atm at 37.8 °C and 90% RH, and 6.58 g H<sub>2</sub>O/100 in<sup>2</sup>/day/atm at 23 °C and 0% RH. A vacuum was created inside the bags using a Multivac C100 (Germany) packaging machine followed by injection with one of three atmospheres: (1) air (passive atmosphere), (2) a mixture of 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> complemented with N<sub>2</sub>, or (3) N<sub>2</sub>. The samples were then sealed and stored at 4 ± 1 °C. Control cladodes (without a modified atmosphere) were placed in ventilated clamshell containers and stored at the same temperature. In order to identify and characterize symptoms of chilling injury, two groups of cactus stems with spines (selected, washed and disinfected as previously indicated) were stored in ventilated clamshells at 23 ± 2 °C, and at 4 ± 1 °C. There were three repetitions per treatment, each consisting of four cladodes, and the assignment of treatments was random.

Quality attributes and fermentation metabolites were determined immediately after harvest and after 5, 10, 15, and 20 days of storage except for weight loss, which was measured at different days throughout storage. In order to evaluate the odor generated in the headspace of MAPs, a second experiment was conducted. Given that in the first experiment the levels of fermentation metabolites were low in all three MAPs evaluated, in this second experiment only one MAP was subjected to analysis of fermentation metabolites, and simultaneously, to odor perception by a trained panel after 7, 14 and 21 days of storage.

**Quality attributes. Color.** In order to evaluate changes in the green color of cladodes, ten disks with a 2.3 cm diameter were obtained from areas where tissue had not been removed during the minimal processing of stems. The color of each disk was measured using a ColorFlex® Model HunterLab colorimeter (Hunter Associates Laboratory, USA). Results were reported using the Hunter color scale parameters: L\* (lightness), a\* (red to green), and b\* (yellow to blue) (HunterLab, 2010).

**Firmness.** The maximum force required to penetrate 7 mm into a cladode's base devoid of cuticle (removed during minimal processing) was determined using a penetrometer (tr®, Italy) equipped with a 5-mm-diameter probe. Results were reported in Newtons (N) (Reid, 2007).

**Titrateable acidity (AT).** This parameter was measured using acid-base titration of cactus stem juice obtained from each repetition with an electrical juicer; 0.1 N of NaOH served as

the titrant and phenolphthalein was used as an indicator. The results were reported in terms of grams of malic acid/100g of fresh cactus stem juice (AOAC, 2000).

**Weight loss.** A digital precision explorer balance (OHAUS Corporation® USA) was used to measure the initial and subsequent variations in weight during storage. Results were reported as the percentage of weight lost compared to initial measurements (Burton, 1982).

**Physiological disorders.** Pitting (small, bronzed sunken areas on the surface of cladodes) was the main symptom observed during storage. In order to evaluate this damage, a five grade categorical scale was created in terms of the area afflicted, where 0 = absence of damage (0% of the surface was afflicted); 1 = light damage (1-5% of the surface was afflicted); 2 = medium damage (6-10% of the surface was afflicted); 3 = moderate damage (11-20% of the surface was afflicted); and 4 = severe damage (>20% of the surface was afflicted) (Fig. 1A). Pitting indices were obtained using these data and the following equation:

$$\sum_{i=1}^{i=n} \frac{(1xn_1) + (2xn_2) + (3xn_3) + (4xn_4)}{n}$$

Where, n<sub>1</sub> = number of cactus stems with a grade of 1, ... n<sub>4</sub> = number of cactus stems with a grade of 4, and n = the total number of cactus stems analyzed.

**Fermentation metabolites.** The cactus stem juice from each repetition was frozen in liquid nitrogen and stored at -70 °C until analysis. In order to quantify the concentrations of acetaldehyde and ethanol in the samples, 6 g of juice were placed in a 30 mL glass vial containing 9 mL of distilled water and 3.6 g of NaCl to favor the release of volatiles from the matrix into the headspace (Harmon, 1997). The vial was then covered with a black viton septum, sealed with a metal ring, and vortexed in a Thermolyne 16700 mixer for 90 s. Solid-phase microextraction (SPME) was used to capture and concentrate the fermentation metabolites of the headspace, using a 75-μ-thick silica fiber coated with Carboxen/polydimethylsiloxane (CAR-PDMS) (Supelco, Bellefonte, CA). The fiber was exposed to the headspace for 10 min, while the vial was incubated in a water bath at 34 ± 1 °C. The volatiles were desorbed from the fiber in a CG Varian 3900 (Varian, Palo Alto, CA) equipped with a flame ionization detector, using a HP-INNOWAX capillary column of 0.32 mm ID, 0.25 μm thickness and 60 m length (Agilent Technologies, USA). The injector and detector temperatures were 150 °C and 260 °C, respectively. The initial oven temperature was 50 °C, which increased at a rate of 10 °C per minute until it reached 130 °C where it remained for 6 min. The fermentation metabolites were identified by comparing their retention times with those of commercial standards, which had been previously extracted, concentrated and analyzed using the same analytical procedure.

Their concentrations were calculated using standard curves of aqueous dilutions of acetaldehyde (Aldrich) and ethanol (J.T. Baker).

**Sensory analysis.** All sensory evaluations were performed in a specially designed room, with eight individual and separated booths. The samples were presented to each panel member through sliding doors in each booth.

*Training of the sensory panel.* The sensory panel consisted of 11 members (8 women and 3 men, 20-60 years old) recruited from among the students and staff of the Universidad Autónoma Metropolitana. The panelists were selected according to the guidelines established in the ISO 8586-1:1993 Standard, and trained once a week for a total of 40 hours in order to develop their ability to identify fermentation metabolites (acetaldehyde and ethanol) and hexanal, a typical compound found in the volatile fraction of cactus stems. Panel members were also trained to generate appropriate odor descriptors of fresh cactus stems according to the methodology of Quantitative Descriptive Analysis (Stone & Sidel, 2004). In the following sessions the panel reached a consensus, correlating the selected volatile compounds used as references with the aforementioned descriptors of cactus stem samples. Using discriminative test, assessors were trained for the correct identification of the attributes by testing the aroma intensity of the three standard volatiles added in known concentrations in the headspace of Polysweat bags (the same type of bags used for MAP) containing fresh cactus stems. For attribute quantization, a 160 mm scale intensity graph (using the markers not perceptible, medium, and high) was developed for training purposes and for evaluating the repeatability (test-retest reliability) of the panelists.

*Sensory tests.* These were conducted at noon. Samples coded with random numbers were kept at room temperature (25 °C) for one hour and then presented in random order to the panelists, together with the standard solutions of the three analyzed volatiles. In each session, the panelists were presented with cactus stems stored in N<sub>2</sub>-MAP at 4 °C and with fresh unstored cactus stems placed in sealed Polysweat bags, as reference. The panelists were then asked to evaluate changes in the intensity of the three aroma descriptors in the headspace of fresh and N<sub>2</sub>-MAP stored cactus stems. A 160 mm unstructured scale (from 0 mm, not perceptible; 80 mm, medium; and 160 mm, highest concentration of the standard) immediately after opening the bags was used by the panelists.

**Statistical analysis.** The XLSTAT program version 2012.1.01 was used for statistical analyses. A multifactorial ANOVA was applied to determine if any significant differences were present among the treatments for each of the variables analyzed, and if any interaction existed with the storage days. When significant differences were detected, a comparison of multiple means was carried out by the method of Duncan (α = 0.05). For weight loss, data were not dependent on

the initial values and were therefore analyzed independently, while in the case of the sensory analysis, data were subjected to a paired t-test (α = 0.05) (O'Mahony, 1985).

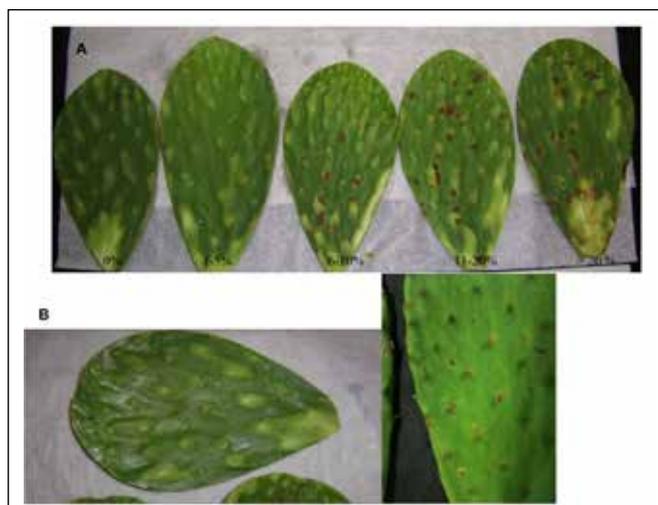
## RESULTS AND DISCUSSION

**Quality attributes of cactus stems.** Initially, all cactus stems possessed good quality attributes as indicated by their fresh appearance, light green color ( $L^*=49.3$ ,  $a^*=-11.99$  and  $b^*=32.2$ ), high titratable acidity TA (1%) and firmness (30.1 N). Statistical analysis showed a significant treatment and storage-time effect in the variables TA,  $L^*$  and  $a^*$ ; only a time effect in firmness and  $b^*$  color parameters; and TA was the only measured variable that showed interactions between treatment and storage time (Table 1).

Similarly to results published by Cantwell et al. (1992), TA decreased during storage in cladodes of all treatments (Fig. 2A) probably because malic acid - the major organic acid in cactus stems - was being consumed as a respiration substrate (Taiz & Zeiger, 2006). It has been reported that MAPs are able to preserve higher acidities in produce. This is because modified atmospheres reduce respiratory rates and thus the consumption of organic acids. As well, the CO<sub>2</sub> present in either the modified atmosphere applied or the one produced as a result of the produce's own respiration acidifies tissues (Kader, 1986; Kader, 2002). However, in the present study only stems preserved in 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> had significantly higher TA values than controls throughout the entire storage period, while the ones preserved in air and N<sub>2</sub> only had higher TA values at 10 and 20 days of storage. This was probably because the former stems were preserved from the beginning of the storage period in a CO<sub>2</sub> enriched atmosphere.

The firmness of cactus stems increased shortly after the beginning of storage (5 d), with MAP-stored cladodes having significantly higher values than controls. Subsequently, however, firmness decreased throughout the entire storage period, with no significant differences found among treatments (Fig. 2B).

$L^*$  values also decreased during storage indicating a shift towards darker colors. This was likely the result of chlorophyll degradation, which renders such dark decomposition products as pheophytin and pheophorbide. However, cactus stems stored in either 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> or N<sub>2</sub> atmospheres were able to maintain their  $L^*$  values practically without change for up to 15 days. Meanwhile,  $a^*$  values increased (i.e., became less negative) while  $b^*$  values decreased during storage, indicating a reduction in green and an increase in yellow colors, respectively. Nevertheless, at 15 days of storage,  $b^*$  values exhibited less changes in stems preserved under N<sub>2</sub> and 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> atmosphere (Fig. 3). Similarly, Guevara et al. (2001) reported that cactus stems (with spines) stored inside MAP (8.6 kPa O<sub>2</sub> and 6.9 kPa CO<sub>2</sub>) at 5 °C preserved their color, while controls changed from a bright green to an opaque green.



**Fig. 1.** Damage scale used to evaluate the surface area affected by pitting (A) and chilling injury symptoms (B), in Atlixco cactus stems stored at  $4 \pm 1$  °C. Left: hydrosis in cactus stems without spines. Right: pitting in cactus stems with spines.

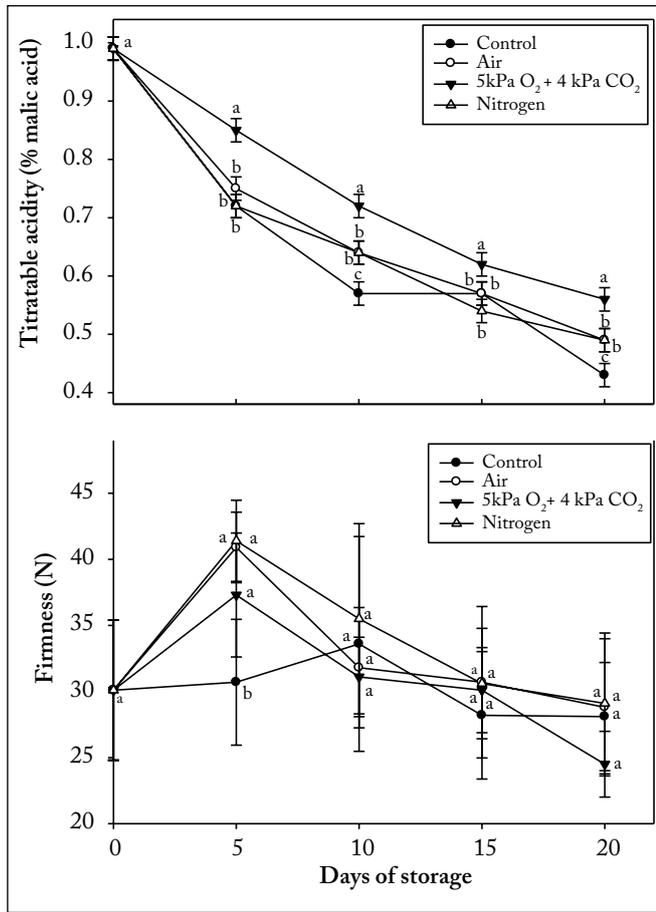
**Fig. 1.** Escala de daño utilizada para evaluar la superficie afectada por picado (A) y daño por frío (B), en nopales de la variedad Atlixco almacenados a  $4 \pm 1$  °C. Izquierda: hidrosis en nopales desespinados. Derecha: picado en nopales con espinas.

The weight loss (WL) of cladodes stored under the three types of modified, study atmospheres was less than 2% after 20 days of storage (Fig. 4). This was a result of the high relative humidity (RH) that accumulated inside packages. This served to limit the transpiration rate of the stems, thereby preventing them from losing their fresh appearance over the course of storage. In contrast, WL in controls was approximately 8% after 20 days (Fig. 5), likely because cladodes in ventilated clamshells were in direct contact with the low RH (70%) of the cold room. As a result, visual signs of weight loss such as stem curling and wilting were readily observed. Rodriguez-Felix & Villegas-Ochoa (1997) reported symptoms of wilting in stems of COPENA F-1 accompanied by a 5% WL, and a 12% WL for COPENA V-1. These results, together with the ones obtained in this study for Atlixco stems confirm that the percentage WL by which the quality of the stems is adversely affected depends on the specific cultivar concerned (Kays, 1997).

Small, bronzed sunken regions as well as areas of moist, translucent tissue appeared on the surface of control cladodes and in those stored inside MAP at 4 °C. The first symptom, identified as pitting, was more frequently present (Fig. 1A), while the second appeared only occasionally and were identi-

**Table 1.** ANOVA of quality attributes for Atlixco cactus stems stored in MAP treatments (air, 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> and N<sub>2</sub>) at  $4 \pm 1$  °C. **Tabla 1.** ANOVA de los atributos de calidad en nopales variedad Atlixco almacenados en EAM (aire, 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> y N<sub>2</sub>) a  $4 \pm 1$  °C.

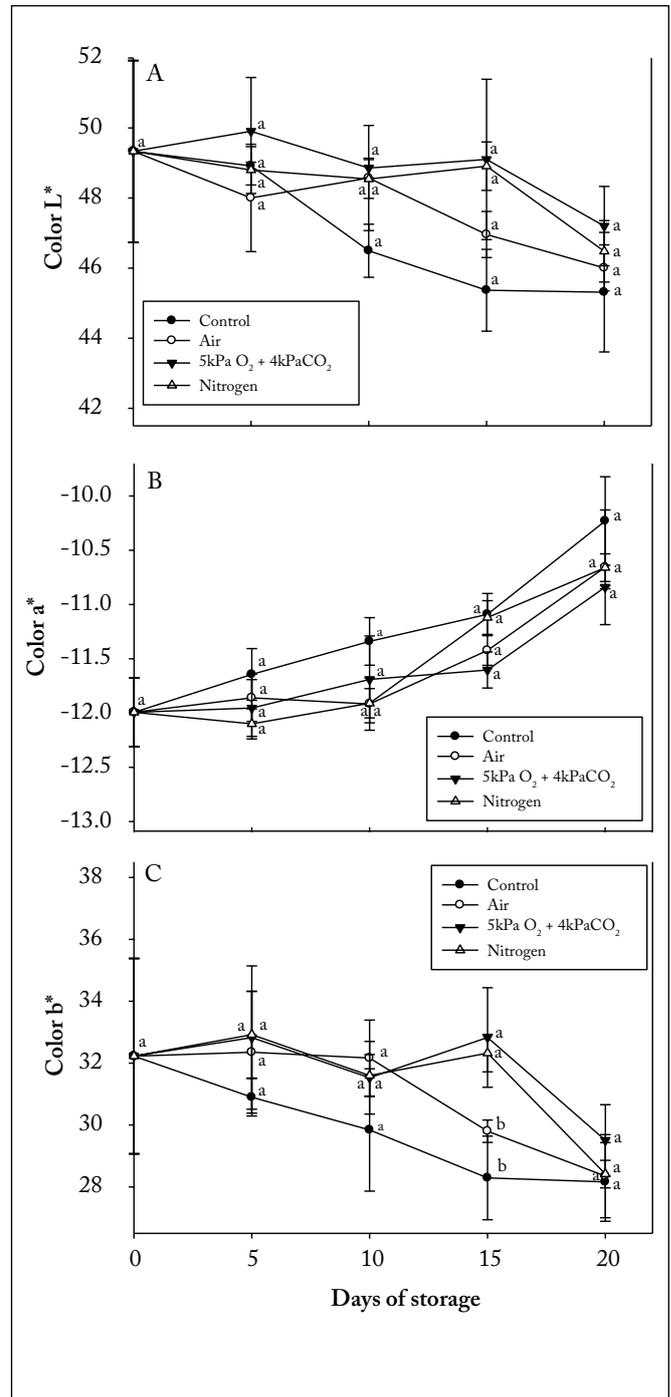
Source	Degrees of freedom	Sum of squares	Mean square	F	Pr> F
<b>Titrateable acidity (%)</b>					
Treatment	5	0.34	0.07	193.02	<0.0001
Day	4	0.74	0.17	527.41	<0.0001
Treatment * Day	13	0.09	0.01	19.71	<0.0001
<b>Firmness (N)</b>					
Treatment	5	122.63	40.87	2.87	0.0528
Day	4	619.31	206.44	14.50	0.0000
Treatment * Day	13	171.54	19.07	1.34	0.2594
<b>Color (L)</b>					
Treatment	5	32.34	10.78	7.99	0.0005
Day	4	39.54	13.18	9.77	0.0001
Treatment * Day	13	15.64	1.74	1.29	0.2837
<b>Color (a)</b>					
Treatment	5	1.45	0.48	7.02	0.0010
Day	4	10.33	3.45	19.87	0.0000
Treatment * Day	13	0.64	0.07	1.03	0.4384
<b>Color (b)</b>					
Treatment	5	37.75	12.58	5.88	0.0028
Day	4	74.04	24.68	11.52	0.0000
Treatment * Day	13	21.32	2.37	1.11	0.3882



**Fig. 2.** Titration acidity (A) and firmness (B) of Atlixco cactus stems stored in MAP at  $4 \pm 1$  °C. Values are the average of three repetitions and the vertical bars represent the standard error ( $n=3$ ). Means with the same letters are not significantly different ( $\alpha=0.05$ ) among treatments.

**Fig. 2.** Acidez titulable (A) y firmeza (B) en nopales de la variedad Atlixco almacenados en EAM a  $4 \pm 1$  °C. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar ( $n=3$ ). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos ( $\alpha=0,05$ ).

fied as hydrosis (Fig. 1B). Similar but less intense symptoms of pitting were observed in the refrigerated cladodes with spines (Fig. 1B) but not on the ones stored at room temperature. This suggests that these symptoms correspond to CI and that Atlixco cactus stems are sensitive to this physiological disorder. The discoloration associated with the observed pitting symptom coincides with the bronzing reported by various authors (Ramayo et al., 1978; Cantwell et al., 1992; Nerd et al., 1997; Cantwell, 2007). These authors also mention other symptoms of CI such as softening or a reduction in firmness values, a susceptibility to diseases, and an increase in respiratory activity and ethylene production. Our results with Atlixco differ from those obtained by our research team in a parallel study of Milpa Alta stems since no CI symptoms were observed in



**Fig. 3.** Hunter color parameters of Atlixco cactus stems stored in MAP at  $4 \pm 1$  °C. Values are the average of three repetitions and the vertical bars represent the standard error ( $n=3$ ). Means with the same letters are not significantly different among treatments ( $\alpha=0.05$ ).

**Fig. 3.** Parámetros de color en nopales de la variedad Atlixco almacenados en EAM a  $4 \pm 1$  °C. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar ( $n=3$ ). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos ( $\alpha=0,05$ ).

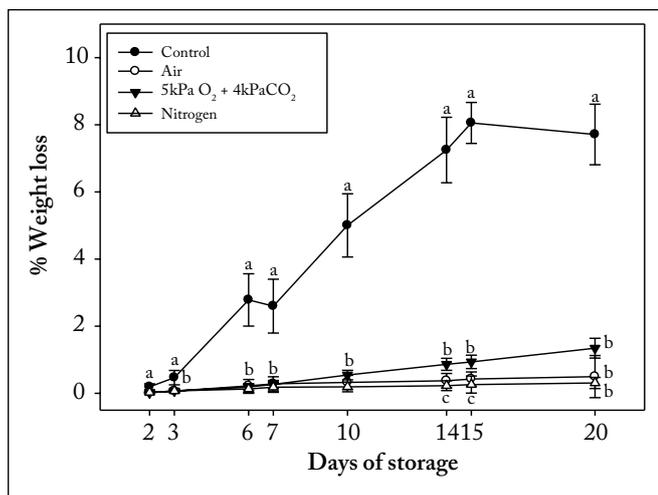


Fig. 4. Weight loss in Atlixco cactus stems stored in MAP at 4 ± 1 °C. Values are the average of three repetitions and the vertical bars represent the standard error (n=3). Means with the same letters are not significantly different among treatments (α=0.05).

Fig. 4. Pérdida de peso en nopales de la variedad Atlixco almacenados en EAM a 4 ± 1 °C. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar (n=3). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos (α=0,05).

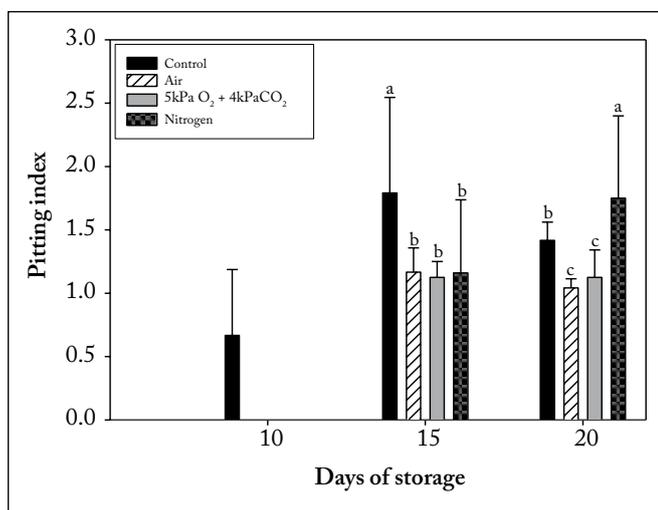


Fig. 5. Pitting index in Atlixco cactus stems stored in MAP at 4 ± 1 °C. Values are the average of three repetitions and the vertical bars represent the standard error (n=3). Means with the same letters are not significantly different among treatments (α=0.05).

Fig. 5. Índice de picado en nopales de la variedad Atlixco almacenados en EAM a 4 ± 1 °C. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar (n=3). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos (α=0,05).

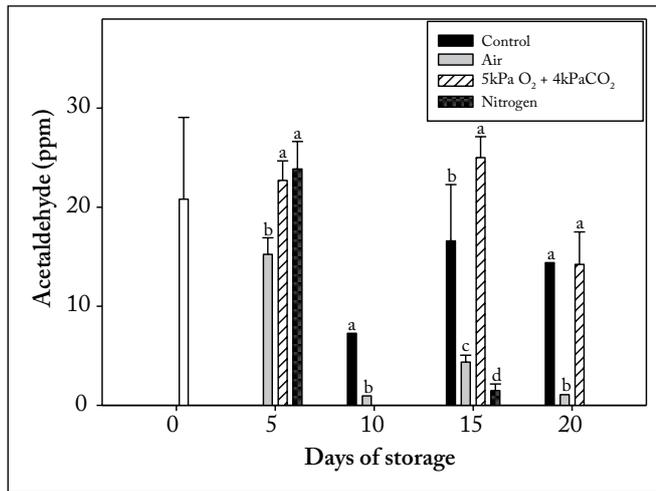
the latter during storage at 4 °C for 25 days (Osorio-Córdoba et al., 2011). This difference is probably due to the fact that the cultivar Atlixco originated in and is commercially produced in warmer regions than Milpa Alta, making it more sensitive to CI. At the same time and unlike Milpa Alta, Atlixco stems did not present browning in areas where spines had been removed, possibly because CI inhibited the cellular response to wounds caused by minimal processing. This response of vegetable tissues, faced with two types of abiotic stresses, where they only respond to one stress while marginalizing the response to the other one, had been previously reported (Saltveit, 2000; Saltveit, 2001; Rivera et al., 2007).

The emergence of CI in the form of pitting, with an index rating >1.0 adversely affected the visual appearance of Atlixco stems; as a result, all stems were regarded as having reached its maximum shelf life at this index rating (>1.0), which corresponds to more than 5% of a cladode's surface being afflicted. Pitting appeared first in controls (after 10 days) and later in MAP-stored stems (after 15 days) (Fig. 5), suggesting that the modified atmospheres applied delayed the onset of CI. This agrees with previous observations made on other produce (Kader, 1986). The pitting index of stems increased throughout storage, reaching values of up to 1.7 in controls and nearly 1.0 in stems stored for 15 and 20 days of storage in either air or 5 kPa O<sub>2</sub> + 4 kPa CO<sub>2</sub> atmospheres. This indicates that such MAPs were able to limit the severity of CI, thus preserving the stems in a commercially-apt condition even after 20 days of storage.

On the other hand, the pitting index of spine-harboring stems stored inside clamshell containers at 4 °C was lower (<0.25) than that of spine-free controls (also preserved in clamshells) after 10 and 15 days of storage. This was likely because, in the former's case, the cuticle protected tissues against the expression of this physiological disorder.

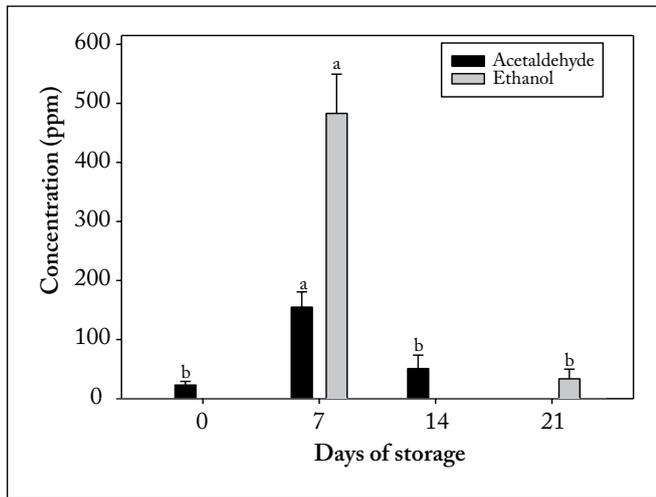
It is worth noting that the incidence of disease was low in all treatments even after 20 days of storage, demonstrating the effectiveness of the control practices used in the organic orchards where the samples were obtained from. The hygienic handling of the cactus stems during both the harvesting operations and the minimal processing applied in this study needs also to be highlighted.

**Fermentation metabolites.** Cactus stems from all treatments had acetaldehyde concentrations ≤30 ppm, and the levels of this compound did not increase during storage (Fig. 6). Similarly, only traces of ethanol were found (<50 ppm) in controls and in stems packaged inside MAP during the entire storage period. This indicates that, for Atlixco cactus stems, fermentation metabolites do not increase in response to modified atmospheres like they do in other vegetable tissues (Dangyang et al., 1994; Kursteiner et al., 2003; Kato-Noguchi & Yasuda, 2007). For example, these tissues include spine-free Milpa Alta stems, where ethanol and acetaldehyde concentrations increased to



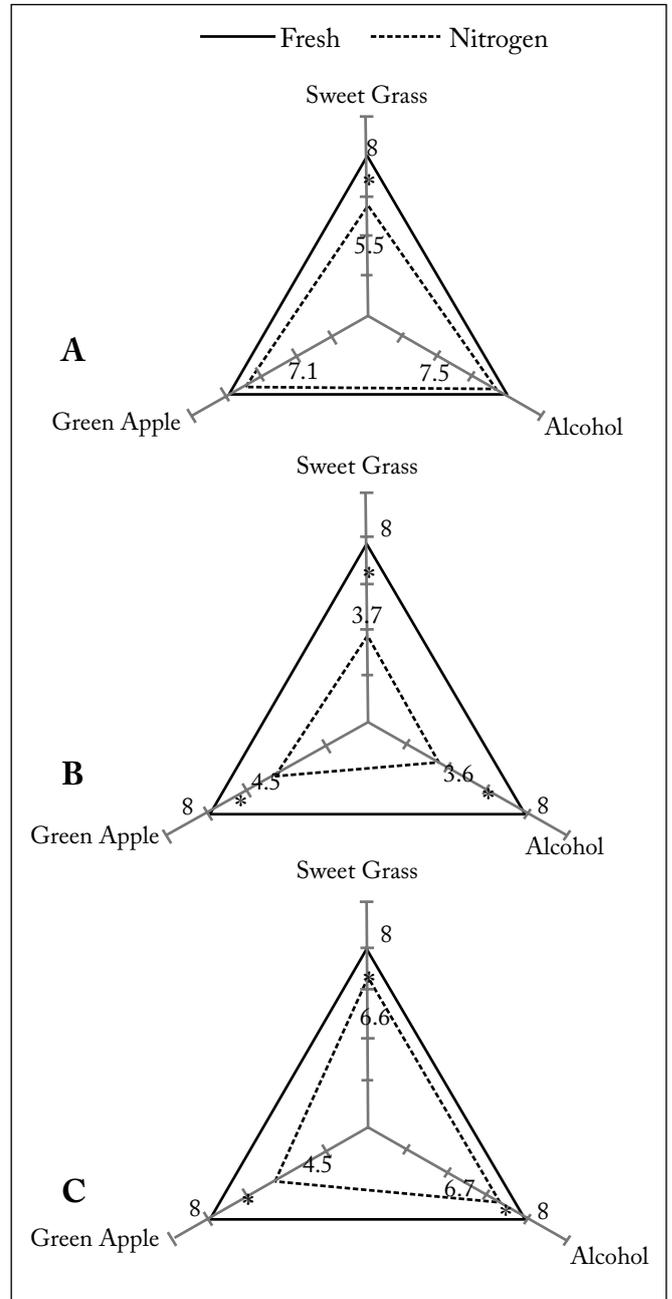
**Fig. 6.** Acetaldehyde levels in Atlixco cactus stems stored in MAP at  $4 \pm 1$  °C. The empty bar shows the initial value. Values are the average of three repetitions and the vertical bars represent the standard error (n=3). Means with the same letters are not significantly different among treatments ( $\alpha=0.05$ ).

**Fig. 6.** Niveles de acetaldehído en nopales de la variedad Atlixco almacenados en EAM a  $4 \pm 1$  °C. La barra vacía muestra los valores iniciales. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar (n=3). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos ( $\alpha=0,05$ ).



**Fig. 7.** Fermentation metabolites of N<sub>2</sub>-MAP Atlixco cactus stems stored at  $4 \pm 1$  °C and subjected to odor perception by a trained panel. Values are the average of three repetitions and the vertical bars represent the standard error (n=3). Means with the same letters are not significantly different among treatments ( $\alpha=0.05$ ).

**Fig. 7.** Metabolitos de fermentación en nopales de la variedad Atlixco almacenados en una EAM de N<sub>2</sub> a  $4 \pm 1$  °C y sometidos a evaluaciones sensoriales por un panel entrenado. Los valores son el promedio de tres repeticiones y las barras verticales representan el error estándar (n=3). Medias seguidas por la misma letra no difieren estadísticamente entre tratamientos ( $\alpha=0,05$ ).



**Fig. 8.** Odor profiles of N<sub>2</sub>-MAP Atlixco cactus stems after 7 (A), 14 (B) and 21 (C) days of storage at  $4 \pm 1$  °C. The values on each axis correspond to the average intensity value of each odor descriptor as evaluated by a trained panel composed of 11 panelists. Descriptors correspond to: sweet herb-acetaldehyde, alcohol-ethanol and green apple-hexanal. \*Means with significant differences at  $\alpha=0.05$ .

**Fig. 8.** Perfiles de olor de nopales almacenados en una EAM de N<sub>2</sub> después de 7 (A), 14 (B) y 21 (C) días a  $4 \pm 1$  °C. Los valores en cada eje corresponden al promedio en la intensidad del valor de cada descriptor de olor evaluado por un panel entrenado compuesto por 11 jueces. Los descriptores corresponden a: hierba dulce-acetaldehído, alcohol-etanol y manzana verde-hexanal. \*Medias con diferencias significativas ( $\alpha=0,05$ ).

250 and 140 ppm, respectively, when stored in MAP (Osorio-Córdoba et al., 2011). A plausible explanation for these results is that the levels of  $O_2$  inside the Polysweat packages was high enough (Fig. 2) to avoid fermentation metabolism in the Atlixco cultivar. In a parallel study conducted by our research group, it was found that the levels of oxygen in air- and  $N_2$ -MAP-stored cactus stems were close to 5% after 15 and 20 days of storage, respectively (Ventura-Aguilar et al., 2013).

**Sensory analysis.** The panellists could not perceive any atypical odor in  $N_2$ -MAP-stored cactus stems. This result was in agreement with the low concentrations of acetaldehyde and ethanol detected in the first experiment (Fig. 6). It also agrees with the low levels of both metabolites that were found after 14 and 21 days in samples of cactus stems that were subjected to sensory analysis in the second experiment (Fig. 7).

The descriptors created from the terms that were suggested by the panellists were: “sweet-herb” for acetaldehyde, “alcohol” for ethanol, and “green apple” for hexanal. The panellists only perceived significant differences in the descriptor “sweet-herb” after seven days of storage, possibly because acetaldehyde was produced in higher quantities by cactus stems at the beginning of this period (Fig. 7). In contrast, after 14 and 21 days of storage, differences were perceived not only in the descriptor “sweet-herb”, but also in the “alcohol” and “green apple” ones as well. Odor profiles, constructed using the values of intensity assigned by the panellists to each of the three descriptors evaluated, showed a reduction in their area after 7, 14 and 21 days of storage (Fig. 8). These results indicate that the intensity values of descriptors as evaluated in the headspace of  $N_2$ -MAP-stored cactus stems were smaller than those of fresh unstored cactus stems placed inside sealed Polysweat bags as reference; however, these changes did not adversely impact the odor perception (absence of atypical odors) of MAP-stored cactus stems.

## CONCLUSIONS

The best response of minimally processed (spine-free) Atlixco cactus stems to MAP preservation was observed in the treatment with 5 kPa  $O_2$  + 4 kPa  $CO_2$ , where the cladodes could be preserved for 20 days at 4 °C while still displaying good quality attributes and allow pitting index (close to 1.0). However, the response of air-MAP- and  $N_2$ -MAP-stored cactus stems in terms of weight loss, pitting index, and overall quality, was acceptable after 15 days of storage at 4 °C. In all three MAPs evaluated, cactus stems produced low levels of acetaldehyde and ethanol. This characteristic conferred a practical advantage to Atlixco stems given that, under modified atmospheres, no off-odors due to the overproduction of acetaldehyde and ethanol could be detected. The odor profile was smaller in MAP-stored cactus than in fresh unstored cactus stems. However, this change didn't have a negative effect

(atypical odors) on sensory perception according to a trained panel. We therefore find that this cultivar is suitable for preservation using the MAP technique.

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