Effects of elevated concentrations of CO₂ in modified atmosphere packaging on the quality of prickly pear cactus stems (Opuntia spp.)

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Abstract

We have tested the effects of passive and semi-active modified atmosphere packaging (MAP) on the postharvest life and quality of flattened stems or cladodes of the prickly pear cactus (Opuntia spp.), called “nopal or nopalitos” in Mexico stored at 5 °C. In semi-active MAP, we injected elevated partial pressures of CO₂ (20, 40 or 80 kPa) in the packages immediately after sealing. Passive MAP (where no CO₂ was added) had an atmosphere of up to 8.9 kPa O₂ and 7 kPa CO₂ after 35 days of storage at 5 °C. Semi-active atmospheres with initial CO₂ pressures of 40 or 80 kPa increased the losses in texture, weight, chlorophyll content, dietary fiber content and color. Passive MAP and semi-active MAP with 20 kPa CO₂ significantly decreased the losses in the above-mentioned parameters, and also decreased the microbial counts (total aerobic mesophiles (AeM), mold and yeasts), but slightly increased the total anaerobic mesophiles (AnM) counts. The microorganisms identified were Pseudomonas, Leuconostoc, Micrococcus, Bacillus, Ruminicoccus, Absidia, Cladosporium, Penicillium and Pichia. Therefore, fresh prickly pear cactus stems can be stored for up to 32 days in MAP with ≤ 20 kPa CO₂ without significant losses in quality nor any significant increase in microbial counts.

Keywords: Opuntia ficus-indica; Microorganisms; Chlorophyll; Chlorophyllase; Fiber

1. Introduction

Nopalitos, the young cladodes of prickly pear cactus stems (Opuntia spp.) are low in calories, high in fiber and traditionally consumed as
vegetable in Mexico. They are commonly ingested as broiled, blended, or as a juice and are used in various pharmaceutical applications for their therapeutic, dermatological and medical properties (Frati, 1992). Experimental evidence demonstrated that the ingestion of some species of nopal (Opuntia streptacantha, O. ficus indica) could decrease both blood glucose and blood lipid levels in patients with non-insulin-dependent diabetes mellitus (NIDDM; Frati et al., 1983). Annual production of prickly pear cactus stems in Mexico is about 400,000 metric tons. They are marketed either as wholes or in slices. The stems are very perishable, being very sensitive to water loss, darkening and decay (Rodriguez-Felix and Soto-Valdez, 1992). Storage life of nopalitos was reported to be 1 day at room temperature and 6 days in refrigerated storage (Rodriguez-Felix and Soto-Valdez, 1992). Modified atmospheres (MA) which involve the decrease of O₂ concentration and/or the increase of CO₂ levels reduce respiration rate, ethylene production and sensitivity, chlorophyll degradation, texture losses, and delay ripening and senescence of several horticultural products (Yahia, 1998). Modified atmosphere packaging (MAP) utilizes polymeric films with selective permeability for O₂, CO₂, C₂H₄, N₂ and H₂O vapor to create an MA around the packaged product. Passive MAP creates a MA due to the respiration of the product and the selective permeability of the packaging material. Semi-active MAP is created by the addition or the removal of a gas mixture immediately after sealing of the package (Kader et al., 1989). MAP reduces or inhibits the growth of food-borne spoilage or pathogenic microorganisms in several types of foods (Daniels et al., 1985; Babic et al., 1996). Elevated CO₂ concentrations decreased the microbial growth in several horticultural products by 100-fold (Gould, 1996). We have observed (Guevara et al., 2001) that passive MAP, where O₂ concentration decreased up to 8.6 kPa and CO₂ concentration increased up to 6.9 kPa, increased the storage life and decreased the quality deterioration of cactus stems stored at 5 °C. The objective of this work was to determine the effects of passive and semi-active MAP (with initial pressures of 20, 40 and 80 kPa CO₂) held at 5 °C for up to 35 days on the storage life and quality of prickly pear cactus stems.

2. Materials and methods

2.1. Plant material

Young cladodes (O. ficus indica, cv. Milpa Alta) were obtained from a commercial plantation in Milpa Alta, Mexico, D.F. The cladodes (about 15 cm long) were harvested manually by cutting the articulation with the “mother cladode” during the early morning, placed in covered carton containers, and transported to the laboratory.

2.2. Processing conditions and treatments

The cladodes were pre-cooled in chlorinated water (100 ppm) at 4 °C for 15 min, and the cut zones were immersed in a solution of ascorbic acid (100 ppm). Cladodes were then left to dry for about 30 min in regular air and classified according to size, uniformity and freedom of defects into five lots of 180 cladodes each. One lot was left without packaging in polymeric bags, packed in a carton box in the same way that cladodes are commonly marketed and was considered as control. The other cladodes were loosely packed in Cryovac RS425 bags (two stems of about 200 g per bag). Cryovac RS425 bags (Cryovac Division, Grace Co., Duncan, SC) had dimensions of 30 cm × 20 cm and a volume of 2 l. The film had a thickness of 0.032 mm, and gas transmission rates for O₂ and CO₂ given by the manufacturer were (1.88–2.82) × 10⁻¹¹ and (8.47–9.40) × 10⁻¹¹ mol m⁻² s⁻¹ Pa⁻¹, respectively. Water vapor transmission was (3.17–5.07) × 10⁻¹² mol m⁻² s⁻¹ Pa⁻¹. One lot was packed in these bags, sealed and considered as passive MAP (without adding CO₂). The third, fourth and fifth lots were placed in the same bags and different pressures of CO₂ (20, 40 and 80 kPa, respectively) were added in each bag immediately after sealing. These were considered as the semi-active MAP treatments. CO₂ was introduced to the packages from a compressed gas cylinder at a pre-determined constant flow through a septum attached to each
package with silicone sealant. Packaged and non-packaged cladodes were held at 5 °C and 80% RH for up to 35 days in the dark.

2.3. Evaluation

Evaluation consisted in the analysis of the concentration of O$_2$ and CO$_2$ in the MAP bags. The parameters evaluated are: weight loss, overall quality scores, color, firmness, dietary fiber content, chlorophyll content (total, a and b), chlorophyllase activity and microbial counts. A sample of 30 cladodes was evaluated for initial quality. A sample of 30 cladodes per treatment (15 bags in the case of packaged cladodes) was removed from storage every 7 days and evaluated.

2.4. Measurement of the in-package atmosphere

In-package atmosphere (O$_2$ and CO$_2$) was measured initially and every 7 days during storage, using a Nitec Model GA-20 portable O$_2$/CO$_2$ analyzer (Nitec, Inc., Cincinnati, OH). The oxygen sensor is an electrochemical fuel cell with a range 0–100 kPa and accuracy of ±0.5 kPa of full scale. The CO$_2$ sensor is a non-dispersing infrared sensor with a range 0–100 kPa and accuracy of ±0.2 kPa of full scale. Gas samples were taken by a syringe from the inside of the package through a rubber septum glued on a surface of the package film. Standards of 9.95 kPa O$_2$ and 9.98 kPa of CO$_2$ (balance N$_2$) were used for calibration of the analyzer.

2.5. Weight loss

Weight loss was determined by weighing the cactus stems at the beginning of the experiment and during each evaluation.

2.6. Overall quality

Overall quality was evaluated on the basis of the following subjective scoring system: 9, excellent quality; 7, good; 5, intermediate; 3, low; and 1, very low. One person on the basis of stem freshness, color and the incidence of decay or other defects did quality evaluation of the cladodes subjectively. Highest quality cladodes are those with dark green color, firm and without an oxidized cut surface. Lower quality cladodes are those with pale green color. The lowest quality cladodes are those with light green color, dark oxidized cut surface, and with possible decay development.

2.7. Color evaluation

Surface color of the stems was measured with a portable Minolta CM-2002 chromometer (Minolta Corp, Osaka, Japan) with illuminant A and 10° viewing angle and calibrated on a white tile, and parameters were determined in a CIELAB system. Measurements were always done at the same marked spot.

2.8. Firmness

The maximum force (N) to rupture pulp tissue was determined with a stable microsystem TA-HD texture analyzer (Texture Technologies Corp, NY) equipped with 5 mm diameter probe penetrating at a velocity of 1 mm/s to a final depth of 5 mm.

2.9. Crude fiber content

Crude fiber content was determined using the neutral detergent reagent method (Van Soest and Wine, 1967). A dry sample (1 g) was mixed with 100 ml of cold laurel sodium sulfate, adjusted to a pH of 6.9–7.1, and mixed with 2 ml of decahydronaphthalene and 0.5 g of sodium sulfite. It was left for 60 min at 110 °C, filtered and washed first with hot water, then with acetone and finally dried on a filter paper for 80 min at 100 °C.

2.10. Chlorophyllase activity

Chlorophyllase (chlorophyll chlorophyllidohydrolase, EC 3.1.1.14) activity was determined by measuring the formation of chlorophyllides (Fernandez-Lopez et al., 1992). The enzymatic extract for activity measurement was obtained by homogenizing 10 g of fresh tissue in 10 ml of cold sodium phosphate buffer (0.2 M) at a pH of 6.5, containing Triton X-100 (5%), PVP (1%) and
cysteine (1 M). The homogenate was centrifuged at 15,700 \( \times g \) for 30 min at 4 °C and filtered through Whatman filter paper No. 2. The reaction medium for measuring chlorophyllase activity contained 1 ml of enzyme extract and 2 ml of substrate (chlorophyll), and activity was determined at 640 nm. The analysis was carried out at 5 °C. Protein content was measured according to the dye binding method of Bradford (1976) using serum albumin for the standard curve. One unit of enzyme activity was defined as the rate of formation of chlorophyllide, relative to the mass of protein.

2.11. Chlorophyll content

Chlorophyll content was determined according to Mencarelli and Saltveit (1988). Fresh tissue (10 g) was homogenized with three aliquots of 15 ml of cold acetone (80%) and vacuum-filtered through a Whatman No. 2 filter paper. Chlorophyll concentration (g kg\(^{-1}\)) was calculated as follows: total chlorophyll \( = 7.12(A_{660}) + 16.8(A_{642.5}) \); chlorophyll \( a = 9.93(A_{660}) - 0.777(A_{642.5}) \); chlorophyll \( b = 17.6(A_{642.5}) - 2.81(A_{660}) \).

2.12. Microbial analysis

Microbiological counts (total aerobic mesophiles (AeM), total anaerobic mesophiles (AnM) and yeast and molds (MY)) were carried out using established methods on 10 g homogenized samples that were macerated in 90 ml of sterile peptone water (pH 7.4; Sneath et al., 1986). Plate count was performed by preparing appropriate sample dilution. Plate count agar (PCA; Oxoid CM463) at pH 7.0 was used for total AeM. Sabouraud dextrose agar (Difco Laboratories, Detroit, MI) was used for MY. Brewer’s thioglycollate medium (Difco Laboratories, Detroit, MI) was used for AnM. All samples were analyzed by triplicate. Samples for AeM and MY were incubated at 34 °C for 24 h, while those for AnM were incubated at 34 °C for 36 h. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. In addition, selectivity of each medium was checked routinely by Gram-staining and microscopic examination of films prepared from randomly selected colonies from all media. Randomly selected colonies from the PCA and Brewer’s thioglycollate agar were identified by biochemical tests according to Sneath et al. (1986) and classified according to their cellular morphology observed by phase contrast microscopy (\( \times 1000 \)), and by their biochemical, physiological and sexual characteristics using the Gram enzymatic test (Cerny, 1976).

2.13. Statistical analysis

The experiment was conducted using a completely randomized design. Statistical analysis was done using SAS for PC (SAS Institute, Inc., Cary, NC) with data subjected to analysis of variance. Color, texture, weight loss and quality data were obtained from 30 independent analyses. Crude fiber, chlorophyll content and enzymatic activity data were obtained from five independent analyses. Microbiological data were obtained from six independent analyses. Values are mean \( \pm SE \).

3. Results and discussion

3.1. Changes in gas composition

Oxygen concentration in the passive MA decreased and reached about 8 kPa after 35 days in storage, while the CO\(_2\) concentration increased to about 7.5 kPa in the same period of time (Fig. 1). In the semi-active MA, where 20 kPa CO\(_2\) was added to the package, the oxygen level decreased to about 13 kPa, and the CO\(_2\) level decreased to about 13.5 kPa after 35 days in storage. On the other hand, the CO\(_2\) level in semi-active MAP with an initial CO\(_2\) level of 40 and 80 kPa, decreased to about 15 and 25 kPa, respectively, and the oxygen level slightly increased to about 15 kPa, at the end of the storage period. The CO\(_2\) levels created in the semi-active MAP with initial of 40 and 80 kPa were higher than the minimum tolerated levels for many fresh horticultural crops (Yahia, 1998).
3.2. Firmness and crude fiber changes

Firmness decreased in all cladodes, but the decrease was faster in those that were kept in semi-active MAP with initial concentrations of 40 and 80 kPa CO₂, and cladodes that were maintained without packaging, and the least firmness loss was observed in the passive MAP and in semi-active MAP with an initial 20 kPa CO₂ (Fig. 2a). The trends in changes in fiber content were similar to those for firmness. The highest loss in fiber was observed in cladodes maintained in semi-active MAP with initial concentrations of 40 and 80 kPa CO₂, and in cladodes that were maintained without packaging, and the lowest loss was observed in the cladodes maintained in passive MAP and in semi-active MAP with an initial concentration of 20 kPa CO₂ (Fig. 2b). It is possible that the loss in firmness is caused by losses in fiber resulting in the softening of the cladodes. The retention in firmness of cladodes maintained in passive MAP or in semi-active MAP with an initial concentration of 20 kPa CO₂ might be due to the positive effects of MA with intermediate CO₂ concentrations. Elevated concentrations of CO₂ were reported to increase firmness losses, to increase cell permeability and to cause a disintegration of wall cell structure (Brecht, 1980).

3.3. Weight loss and overall quality

Weight loss was least in cladodes that were packaged in passive MAP and in semi-active MAP with an initial concentration of 20 kPa CO₂, and the rest of the conditions showed a high weight loss of more than 25% (Fig. 3a). There was a close relation between weight loss and the overall quality (Fig. 3b). Cladodes that were not packaged lost their brilliant green color appearance and became dull as the storage period increased. Similar results were found in lettuce (Brecht et al., 1973; Lipton, 1977). Overall quality of the
cladodes as judged subjectively was highest in cladodes that were packaged in semi-active MAP with an initial 20 kPa CO₂, followed by those packaged in passive MAP. Levels ≤ 20 kPa CO₂ can decrease the rates of respiration and transpiration, resulting in low weight loss. Quality deterioration of shredded lettuce, which included the deterioration in texture, color and odor, was delayed in storage at 5 kPa CO₂ (Kakiomenou et al., 1996). On the other hand, high CO₂ concentrations (over 20 kPa) resulted in changes in cell permeability and increased metabolic processes. Elevated CO₂ concentrations inactivate glycolytic and tricarboxylic enzymes (malate dehydrogenase, succinic dehydrogenase and cytochrome oxidase), generate an accumulation of succinic acid, malate, acetaldehyde, ethanol, and reduce cytochrome activity (Brecht, 1980; Kader, 1980).

**3.4. Color changes**

The L* value (Fig. 4a) slightly increased in cladodes maintained in semi-active MAP with initial concentrations of 40 and 80 kPa CO₂, and in those maintained without packaging, for up to 25 days in storage. The lowest L* values were observed in cladodes packaged in passive MAP and in semi-active MAP with an initial concentration of 20 kPa CO₂. The a* value for the first 25 days in storage (Fig. 4b) slightly increased in all treatments as a result of the loss in green color. However, the increase was slower in cladodes packaged in semi-active MAP with initial partial pressures of 40 and 80 kPa, and in cladodes that were not packaged. By the end of storage, cladodes packaged in semi-active MAP with initial partial pressures of CO₂ at 40 and 80 kPa had the highest a* values and controls, the lowest. The b* value (Fig. 4c) increased, and the highest increase was
observed in cladodes packaged in semi-active MAP with an initial 80 kPa CO₂, followed by those maintained in semi-active MAP with an initial 40 kPa CO₂, and by those kept without packaging until 25 days in storage, after which a decrease was observed. Darkening was present in cladodes packaged in semi-active MAP with an initial of 40 and 80 kPa CO₂, especially during the last period of the study, which might be due to enzymatic oxidation. External color of cladodes that were not held in MAP changed from brilliant green to dark green. The increase in L* value in cladodes kept without packing and in those maintained in semi-active MAP with initial pressures of 40 and 80 kPa CO₂ for up to 25 days in storage indicates a decrease in the dark green color (increase in lightness), which is in agreement with the increase in chlorophyllase activity and decrease in chlorophyll content. However, the decrease in L* value in cladodes kept in semi-active MAP with initial pressures of 40 and 80 kPa CO₂ is due to darkening of the tissue, which might be due to oxidative reactions due to high CO₂ injury. The same tendency was shown in the b* value, which indicates an increase in yellowing in non-packed cladodes and in cladodes maintained in semi-active MAP with initial pressures of 40 and 80 kPa CO₂ for up to 25 days in storage, followed by a decrease in b* indicating the darkening of the tissue caused by the high levels of CO₂. Changes in a* and b* values were observed in broccoli held in elevated CO₂ concentrations (Wang, 1979).

3.5. Chlorophyll content

The decrease in chlorophyll content (total, a and b) (Fig. 5) during most of the storage period was the least in cladodes that were maintained in passive MAP and in semi-active MAP with an initial of 20 kPa CO₂, followed by those packaged in semi-active MAP with initial concentrations of 40 and 80 kPa CO₂, and in those kept without packaging. Total chlorophyll and chlorophyll a of cladodes kept in passive MAP and in semi-active MAP with 20 kPa CO₂ decreased about 23 and 35%, respectively, while elevated CO₂ concentrations (40 and 80 kPa) caused a decrease of about 53 and 63%, respectively (Fig. 5a and b). Chlorophyll b content decreased about 57, 58, 37, 80 and 99% in cladodes that were not packaged, packaged in passive MAP, or packaged in semi-active MAP with initial 20, 40 or 80 kPa CO₂, respectively, after 35 days in storage (Fig. 5c). Chlorophyll a was higher than chlorophyll b at all stages. However, their degradation rate was similar. Chlorophyll loss was reported to be low in vegetables stored in elevated CO₂ environments (Shewfelt et al., 1983). The degradation of chlorophyll in broccoli florets was accelerated by ethylene and suppressed by controlled atmospheres (Yamauchi and Watada, 1993).

3.6. Chlorophyllase activity

Chlorophyllase activity increased in all cladodes as the storage period increased (Fig. 5d). The lowest activity was present in cladodes packaged in passive MAP and in semi-active MAP with 20 kPa CO₂, and the highest activity was found in cladodes packaged in semi-active MAP with an
initial concentration of 40 and 80 kPa CO₂, and in cladodes kept without packaging. The highest chlorophyllase activity present in treatments with elevated CO₂ concentrations (semi-active MAP with initial 40 and 80 kPa) is probably due to the stress caused by CO₂ injury. There is a clear relation between chlorophyll degradation and chlorophyllase activity. Conditions that showed the highest chlorophyll degradation also had the highest chlorophyllase activity. This is in disagreement with Baardseth and Von Elbe (1989) who concluded that gradual chlorophyll disappearance in spinach did not coincide with the activation of chlorophyllase. The chlorophyllase activity was found to either increase (Rodriguez et al., 1987) or decrease (Majumdar et al., 1991) during the senescence of leaves and ripening of fruit. This suggests that chlorophyllase may or may not be responsible for chlorophyll degradation. However, it seems likely that chlorophyllase is important for chlorophyll degradation in prickly pear cactus stems.

3.7. Microbiological analysis

Microbial population started to increase after 15–20 days of storage (Fig. 6). The least increase in AeM population was in cladodes that were kept in semi-active MAP with an initial of 20 kPa CO₂, followed by those maintained in semi-active MAP with an initial concentration of 40 kPa CO₂, and the highest increase was in cladodes maintained in semi-active MAP with an initial of 80 kPa CO₂, and in cladodes that were maintained without packaging (Fig. 6a). AeM population reached up to $1.9 \times 10^5$ CFU g⁻¹ after 35 days in storage. AnM (Fig. 6b) were highest in cladodes that were held in semi-active MAP with an initial of 80 kPa CO₂ and least in those held without packaging and in those held in semi-active MAP with an initial of 20 kPa CO₂. The highest AnM count was $2.0 \times 10^5$ CFU g⁻¹. The highest increase in MY (Fig. 6c) was found in cladodes that were held in semi-active MAP with an initial of 40 kPa CO₂, followed by those maintained without packaging, while the least MY population was in cladodes maintained in semi-active MAP with initial concentrations of 20 and 80 kPa CO₂. The highest MY population reached up to $5 \times 10^4$ CFU g⁻¹.

Isolation of single colonies was performed by randomly selecting the square root of the total number of colonies counted on each plate. Identification was conducted according to Bergey's manual (Sneath et al., 1986). On the basis of the macro- and microscopic characteristics of AeM bacteria isolated and the biochemical test done, we identified bacteria of the genus Leuconostoc, Pseudomonas, Micrococcus and Bacillus. The AnM were identified in the same way as the AeM, and the bacteria found were of the Ruminococcus genus. In the case of MY, we took into consideration the reproduction and hyphae form and macroscopic and selective growth, and on that basis we have identified the fungi Absidia, Cladosporium and Penicillium and the yeast Pichia, which produces necrosis of tissue.

Microbial counts were low compared to those encountered in other fresh horticultural products.
Mesophilic aerobic bacterial counts were found to be $10^7$ CFU g$^{-1}$ in fresh cut spinach (Babic et al., 1996). Yeast has been reported in the microbial flora of minimally processed vegetables (Albrecht et al., 1995). The effect of CA on microorganisms is temperature-dependent, with the inhibitory effect being less at higher temperatures. CO$_2$ was reported to inhibit some types of microorganisms but has no effect on others (Hintlian and Hotchkiss, 1986). CO$_2$ under pressure inhibited bacteria, mold and yeast (Haas et al., 1989). Nguyen-The and Carlin (1994) reported that elevated CO$_2$ concentrations significantly reduced the development of mesophilic bacteria on chichory leaves at 2 and 6°C but had no effect at 10°C. Elevated CO$_2$ atmospheres also had an inhibitory effect on the growth of aerobic microorganisms on broccoli kept at 4°C (Berrang et al., 1990). The bacteriostatic effect of CO$_2$ is not fully understood, but it could be due to lowering of the pH of growth media, the exclusion of O$_2$ by replacement of CO$_2$ or the acidification effect of CO$_2$ (Daniels et al., 1985), or decreasing the O$_2$ availability (Babic et al., 1996). It has been reported that cladodes can be infected by various fungi, namely Colletotrichum gloeosporioides, which produce round black necrosis under high humidity (Fucikovski, 1992). Phytophthora cactarum and P. omnivera can cause wilt and possible rot (Cacioppo, 1991), and Phillosticta opuntia can cause certain type of scab. Bacteria of coliform group, with isolates similar to Erwinia chrysanthemi and E. carotovora subsp. atrosepica were identified as agents of soft rots in cladodes by Fucikovski and Jaimes (1981) and Vavaro and Gargata (1990).

4. Conclusions

It is possible to extend the shelf life of prickly pear cactus stems. This can be achieved by generating an atmosphere with O$_2$ levels of up to 8 kPa and CO$_2$ levels of up to 7 kPa in passive MAP, or up to 20 kPa in semi-active MAP. CO$_2$ concentrations between 7 and 20 kPa decreased the loss in color, firmness and fiber content, and reduced chlorophyllase activity, and microbial flora load on the stems. These benefits are due to atmospheric modification and not to increased humidity in the atmosphere (Guevara et al., 2001). On the other hand, elevated CO$_2$ levels ($\geq 40$ kPa) caused injury in cladodes in comparison with non-packaged cactus stems. There were no big differences in the quality of prickly pear cactus stems packaged in passive MAP or in semi-active MAP with an initial CO$_2$ concentration of 20 kPa. The relative limit of tolerance of prickly pear cactus stems to CO$_2$ was 20 kPa. The storage life of the cactus stems can be up to 32 days in passive MAP or in semi-active MAP with an initial CO$_2$ concentration of 20 kPa. Bacteria of the genus Leuconostoc, Bacillus, Pseudomonas, Micrococcus and Ruminicoccus were identified in the microflora of O. ficus indica cactus in MAP. The molds isolated were of the genus Absidia, Cladosporium, Penicillium, in addition to the yeast Pichia. No pathogenic microorganisms were identified in the cactus stems.

References


