Cold Storage Effects on Quality of Parthenocarpic and Pollinated Prickly Pear (*Opuntia ficus-indica* Mill.) fruits

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Abstract. Prickly pear (*Opuntia* spp.) is a fruit with high nutritional and functional value, but it is highly perishable. Parthenocarpic cactus pear fruits (CP30-P, CP40-P) are a viable commercial alternative because they have almost imperceptible seeds and longer shelf life. However, their response to cold storage, compared to pollinated prickly pear fruits, has not been reported. This study evaluated the effect of cold storage at $10 \pm 1\,°C$ and $85 \pm 2\%$ relative humidity on the quality of parthenocarpic (CP30-P and CP40-P) and pollinated (CP30 and CP40) prickly pear fruits. The variables such as weight loss, total soluble solids (°Brix), acidity, antioxidant activity, electrolyte leakage, and enzymatic activity (CAT and APX) were assessed during cold storage. The parthenocarpic fruits had higher weight loss compared to pollinated fruits (15% vs. 8%), greater electrolyte leakage, and lower catalase activity, indicating increased susceptibility to water loss and altered cell membrane permeability. However, these fruits also exhibited higher antioxidant activity at 30 and 45 days, suggesting a physiological response to oxidative stress induced by cold storage. No significant changes were observed in total soluble solids or titratable acidity, demonstrating that these parameters remained stable throughout the study. Cold storage is an effective strategy to preserve the postharvest quality of prickly pear, particularly regarding composition stability. Nevertheless, parthenocarpic fruits require additional strategies to minimize dehydration and improve resistance under prolonged storage conditions.

Keywords: permeability, stress, membrane, quality, edible

Introduction

Plants of the genus *Opuntia*, adapted to arid and semi-arid environments, represent a promising alternative to diversify fruit production in regions with limited water availability (Heyduk, 2022; Zhang *et al.*, 2024). The fruits of *Opuntia ficus-indica* Mill. have high nutritional and functional value, such as vitamins K (53.2-64.2 mg 100 g^{-1}), C (28-79.2 mg 100 g^{-1}) and E (527.4-623.2 mg 100 g^{-1}), in addition to minerals such as potassium (199-410.7 mg 100 g^{-1}), calcium (12.4-49.1 mg 100 g^{-1}) and magnesium (18 mg 100 g^{-1}), as well as amino acids, antioxidant compounds and dietary fiber (5.83%) (Sabtain *et al.*, 2021; Loukili *et al.*, 2024). However, the marketing of tuna still faces significant limitations, due to the presence of large and hard seeds that affect consumer acceptance, and its short shelf life that restricts distribution to more distant markets (Livera *et al.*, 2023).

Parthenocarpic fruit development has emerged as a promising approach to overcome limitations associated with seed presence and postharvest losses, as it reduces or eliminates seeds and can extend fruit shelf life. In tomato (*Solanum lycopersicum*), parthenocarpic fruits show extended shelf life compared to seeded fruits, a trait linked to higher gibberellin levels and the up regulation of biosynthetic genes such as GA3ox1 and GA20ox, which are associated with delayed senescence (Dominic *et al.*, 2021; He *et al.*, 2021; Sharif *et al.*, 2022). Morphologically, parthenocarpic fruits may present a thicker pericarp and larger parenchymal cells, which could enhance resistance to chilling injury (Zhang *et al.*, 2023; Niu *et al.*, 2024). Nevertheless, these fruits are often smaller than pollinated counterparts and exhibit larger pericarp cells arranged vertically, potentially increasing exposed surface area and water loss during storage (Niu *et al.*, 2024; Flores-Hernández *et al.*, 2025).

In parthenocarpic fruits of the *Opuntia* genus, the pericarpel exhibits a distinct tissue organization, characterized by thinner cell walls, lower cell compaction, and wider intercellular spaces. Similar structural features have been observed in parthenocarpic atemoya (Annona × atemoya), where cytological studies reported increased cell expansion, vacuolization, and reduced calcium accumulation, traits associated with lower fruit firmness (dos Santos *et al.*, 2019; Flores-Hernández *et al.*, 2025). These anatomical characteristics indicate that parthenocarpic fruits differ from pollinated fruits in their physical and structural properties, which may influence their postharvest behavior and susceptibility to environmental stresses. These characteristics make parthenocarpic fruits more susceptible to chilling injury (Corrales-García *et al.*, 1997), manifested as dehydration, browning, and cell collapse (Zhang *et al.*, 2023; Sabir *et al.*, 2024). At the physiological level, this phenomenon is related to the loss of membrane integrity, increased oxidative stress, and cell wall disorganization, processes widely described in fruits and vegetables (Wu *et al.*, 2024). During cold storage, the accumulation of reactive oxygen species (ROS) typically activates enzymatic antioxidant systems, such as catalase (CAT) and ascorbate peroxidase (APX), which remove hydrogen peroxide and protect cellular structures (Gill and Tuteja, 2010; Sharma *et al.*, 2012). However, in parthenocarpic fruits, these enzymes have been observed to exhibit lower activity, limiting their detoxification capacity and exacerbating oxidative damage (Zhao *et al.*, 2009). These characteristics represent a limitation for cold storage, even though their advantages such as greater uniformity and an edible pericarpel of commercial interest (Flores-Hernández *et al.*, 2024).

At room temperature (21 ± 1 °C, 61 ± 2% relative humidity), prickly pear fruits have a shelf life of 9-15 days, whereas parthenocarpic ones can last up to 30 days. However, there are no studies comparing the response of these fruits to low-temperature storage (Cruz-Bravo *et al.*, 2019; Flores-Hernández *et al.*, 2025). Therefore, the aim of this study was to evaluate the response to cold storage and enzymatic activity of pollinated and parthenocarpic prickly pear fruits from two varieties.

Material and Methods

Study site and plant material

Pollinated prickly pear fruits of the CP30 (yellow) and CP40 (red) varieties and their parthenocarpic counterparts (CP30-P and CP40-P) were harvested at horticultural maturity from 8-year-old plants in an experimental orchard in the State of Mexico, Mexico. Parthenocarpic fruits were obtained following the methodology reported by Livera *et al.* (2023). The fruits were harvested when they exhibited the characteristic maturity pigmentation of each variety across the entire peel, collecting 50-60 fruits per treatment. During harvest, a portion of the cladode was included to promote efficient healing and

prevent stem rot. The glochidia were removed using a dethorner, and the fruits were subsequently placed in plastic boxes and transferred to the postharvest physiology laboratory.

The fruits were selected for the absence of damage and disease and washed with a 0.01% sodium hypochlorite solution. They were stored at 10 ± 1 °C and 85 ± 2% relative humidity for 45 days. For weight loss measurements, 10 fruits per treatment were labeled and recorded every five days. All other variables titratable acidity, total soluble solids, antioxidant capacity, electrolyte leakage, and enzymatic activities of ascorbate peroxidase (APX) and catalase (CAT) were also measured every five days, considering one fruit as a replicate, with a total of five replicates per treatment. In the results, only data from days 0, 30, and 45 are presented as values obtained between days 0 and 30, and between days 30 and 45, were practically identical.
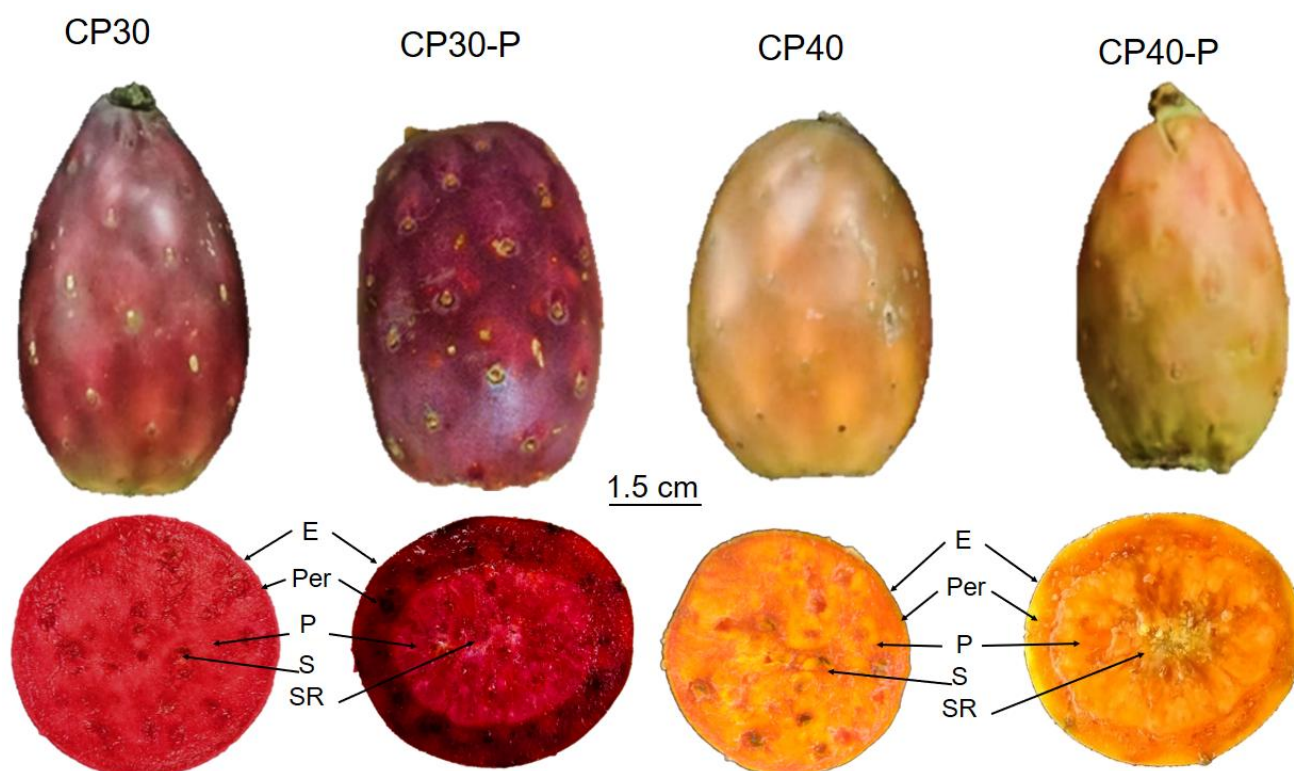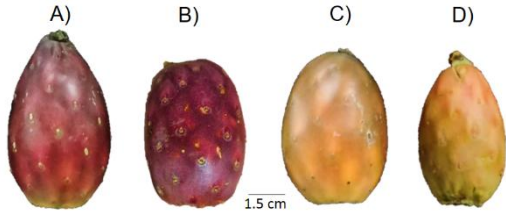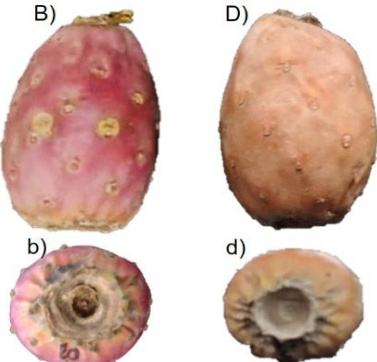

Figure 1. Appearance and cross-section of the prickly pear varieties (*O. ficus-indica* L. Mill). CP30: Pollinated fruit, CP30-P: Parthenocarpic fruit, CP40: Pollinated fruit, CP40-P: Parthenocarpic fruit. E: Epidermis; Per: pericarpel (peel); P: pulp; S: seed; SR: Seminal residue.

Chilling injury
Changes in fruit appearance and dehydration were monitored during the storage period, following the methodology reported by Ochoa and Guerrero (2012). To quantify dehydration, a visual scale of turgor loss and fruit shrinkage was established, allowing standardized classification of the degree of dehydration (Table 1).

Table 1. Dehydration scale during cold storage (10 ± 1 °C and 85 ± 2% RH) of *Opuntia ficus-indica* Mill. fruits.

Level	Dehydration	Fruit appearance
0	No dehydration: Fresh and firm fruit, smooth and shiny surface without signs of water loss. WL: 0-2 %	
1	Mild: Slight weight loss, not affecting commercial quality. Pericarpel is less glossy, with no visible wrinkles. WL: 3-7 %	
2	Moderate: Evident weight loss, beginning to affect commercial quality. Dull pericarpel, slight dehydration of the pericarpel, except in pollinated fruits, which show fungal incidence. WL: 8-13 %	
3	Severe: Advanced dehydration in the hypanthium area, pronounced dehydration of the pericarpel. WL: 14-25 %	

A) CP30: Pollinated; B) CP30-P: Parthenocarpic; C) CP40: Pollinated; D) CP40-P: Parthenocarpic; b) Hypanthium of CP30-P; d) Hypanthium of CP40-P; WL: Weight loss.

Weight loss
The weight was determined in 10 fruits using a digital balance with 0.01g precision (Ex2200 Alsep®, A&D Company, Tokyo, Japan). The same fruits were weighed every five days until the end of storage. The weight loss was expressed as a percentage, using the initial weight as a reference.

Electrolyte leakage
Three 1-cm diameter discs were excised from the middle of the pericarp of each fruit, with one fruit considered as a single replicate, resulting in a total of five replicates. The discs were placed in a flask

containing 30 mL of 4 mM mannitol solution and allowed to stand for three hours. Total dissolved solids (TDS) were then measured using a PM-3000 refractometer (Liquatec). The flasks were subsequently heated in a water bath for 1 hour, and TDS was measured again (Soleimani *et al.*, 2015). Electrolyte leakage was calculated as a percentage according to the formula described by Promyou *et al.* (2012).

$$\text{Electrolyte leakage (\%)} = \frac{\text{Initial electrolyte leakage}}{\text{Final electrolyte leakage}} \times 100 \qquad [1]$$

Catalase (CAT)
Acetone powder was prepared from five fruits, one fruit was considered as a single replicate, resulting in five replicates. For this purpose, 10 g of pericarp from each fruit were ground with cold acetone, repeating the process three times, the powder then was dried and stored at −80 °C in an ultra-low temperature freezer to preserve enzymatic activity. The catalase is relatively stable under these conditions and resistant to partial degradation during acetone extraction. The catalase (CAT) activity was analyzed by mixing 0.1 g of acetone powder with 5 mL of TRIS-HCl buffer (0.1 M, pH 8.5) containing 2% polyvinylpyrrolidone (PVP), followed by centrifugation at 4000 × *g* and 4 °C for 30 min. Then, 200 µL of the supernatant was mixed with 200 µL of H_2O_2 (0.2%) and 2 mL of TRIS-HCl buffer (10 mM, pH 8.5). Absorbance was recorded every 10 s for one minute at 240 nm using a spectrophotometer (Genesys 10s UV-Vis®, Thermo Spectronic, Madison, WI, USA) (Martínez-Damián *et al.*, 2013). The results were expressed as U g^{-1} fresh pericarp.

Ascorbate Peroxidase (APX)
The enzyme activity was measured by taking 0.1 g of acetone powder, adding 5 mL of phosphate buffer (50 mM, pH 7.8) plus 0.2 mM EDTA (ethylenediaminetetraacetic acid) (Sigma-Aldrich, São Paulo, Brazil) and 2% PVP (polyvinylpyrrolidone) (Sigma-Aldrich, São Paulo, Brazil). The samples were centrifuged at 4000 × *g* at 4°C for 30 min, then 200 µL of the supernatant was taken and mixed with 200 µL of H_2O_2 (0.2%) and 2 mL of phosphate buffer (50 mM, pH 7.0) (Zhang *et al.*, 2013). The absorbance was recorded every 10 s for one minute at 290 nm in a spectrophotometer (Genesys 10s UV-Vis®, Thermo Spectronic, Madison, WI, USA). The results are expressed in U g^{-1} FW.

Antioxidant Capacity
The method of Kim *et al.* (2002) with some modifications was followed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich; St. Louis, MO, USA). This was determined in five fruits. The violet colored DPPH turns yellowish upon contact with the antioxidant sample, and readings were taken at 517 nm on a spectrophotometer (Genesys 10 UV-Vis, Thermo Spectronic®, Madison, WI, USA) at 10, 20, 30, and 60 min. Antioxidant capacity was represented as radical scavenging capacity (% RSC) with the absorbance of DPPH without sample at time zero being 100%.

Titratable Acidity and Total Soluble Solids
Acidity was determined using the volumetric method (AOAC, 1990). Ten g of tissue per fruit was weighed and liquefied with 50 mL of distilled water. A 5 mL aliquot of the mixture was taken, and three drops of phenolphthalein were added as a color indicator. The mixture was then titrated with NaOH (0.01 N) to pH 8. The results were expressed as % citric acid. Total soluble solids were determined in five fruits by weighing 5 g of the middle portion of each fruit, from which the juice was extracted, and

placing two drops in a digital refractometer (PAL-1, ATAGO®, Tokyo, Japan). The result was expressed as °Brix (AOAC, 2023).

Statistical Analysis

A completely randomized design was used, each variety as treatments and a replicate consisting of one fruit. CP30 and CP30-P fruits were compared with each other, and the same was conducted for CP40 and CP40-P fruits. Data were expressed as mean ± standard deviation, and the analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$) were also performed. All analyses were performed with SAS software (On Demand for Academics® version 9.04) (SAS, 2023), and graphs were obtained with GraphPad Prism software version 7.00.

Results and Discussion

Chilling injury and weight loss

Table 1 shows the damage caused by cold storage, reflected in changes in fruit appearance and weight loss. Parthenocarpic fruits exhibited significant weight loss at scale 2 (moderate: 8-13%), indicating initial signs of dehydration and loss of surface gloss. At this stage, notable dehydration occurred in the hypanthium and pericarpel areas. In contrast, pollinated fruits showed a higher incidence of disease. Overall, parthenocarpic fruits experienced greater weight loss, reaching 15% at the end of storage (45 d), while pollinated fruits lost an average of 8% (Figure 2). The lower weight loss observed in pollinated fruits suggests better water retention, likely to be associated with a more efficient cellular structure.
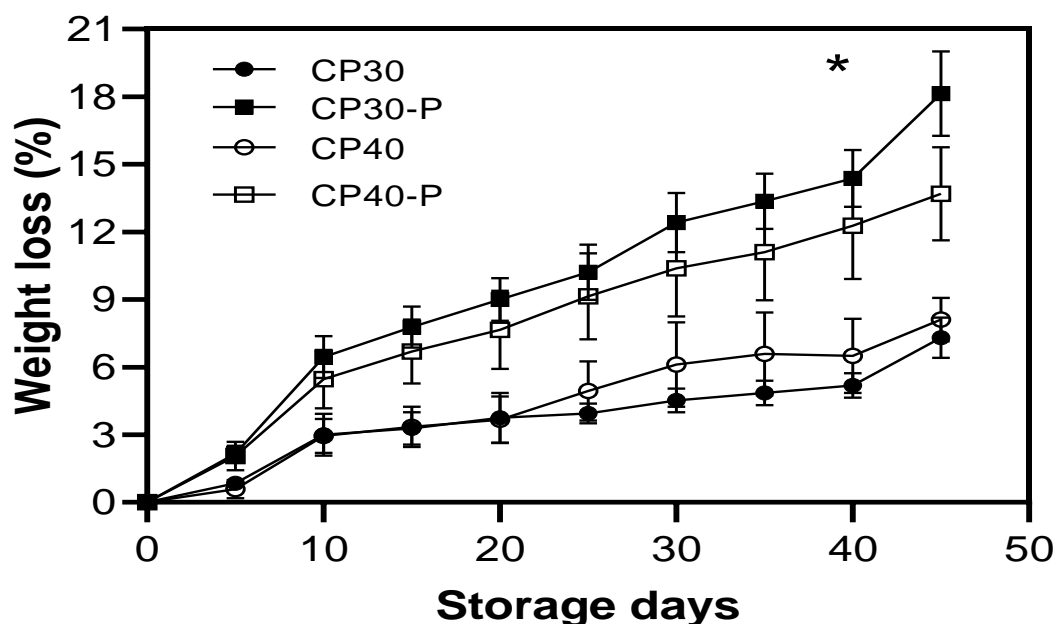
Figure 2. Weight loss during cold storage (10 ± 1 °C and 85 ± 2% relative humidity) of *Opuntia ficus-indica* Mill. fruits. CP30: Pollinated; CP30-P: Parthenocarpic; CP40: Pollinated; CP40-P: Parthenocarpic. WL: Weight loss. Values represent means ± SD (n = 10). *Indicates a statistically significant difference between parthenocarpic and pollinated fruits of the same variety.

Electrolyte Leakage and Enzymatic Activity

Electrolyte leakage is an indicator of cell membrane damage. It is primarily caused by the efflux of K+ and counterions (Cl^{-1}, HPO_4^{-2}. NO_3^{-1}, $citrate^{-3}$, $malate^{-2}$) that move to balance the efflux of positively charged potassium ions. Increased electrolyte leakage means that cell membranes are more permeable (Saebi *et al*., 2023). Electrolyte leakage progressively increased during storage in all evaluated varieties (Table 2). During the first 30 days, both parthenocarpic and pollinated fruits showed leakage values from 50 and 57%, with no statistically significant differences. After this period, further variations were observed, so that at the end of storage (45 d), an average increase of 14% was recorded across all varieties. At the end of storage, parthenocarpic fruits showed significantly greater electrolyte leakage than pollinated fruits; this increase was observed substantially from day 30, possibly due to senescence processes. Sedaghathoor *et al*. (2023) reported that higher membrane permeability and ion leakage rates are inversely correlated with the duration of cold storage, as prolonged exposure to low temperatures induces structural alterations in the lipid bilayer. During this process, phospholipids undergo a phase transition that reduces membrane fluidity, while the accumulation of reactive oxygen species causes lipid peroxidation and damage to transport proteins. These modifications reduce the membrane's ability to maintain cellular integrity, promoting non-selective electrolyte passage and, consequently, an increase in ion leakage as storage progresses.

Table 2. Electrolyte leakage, CAT and APX activity during cold storage (10 ± 1 °C and 85 ± 2% RH) of *Opuntia ficus-indica* Mill. fruits.

Evaluation day/Variety	Electrolyte leakage (%)	Catalasa (U g⁻¹ FW)	Ascorbate peroxidase (U g⁻¹ FW)
0 storage days			
CP30	50.5 ± 3.3	21.0 ± 3.2	35.8 ± 2.7 a
CP30-P	57.3 ± 2.5	22.1 ± 2.6	31.4 ± 2.2 b
CP40	52.9 ± 1.6 b	24.4 ± 4.5 a	40.7 ± 2.9
CP40-P	56.3 ± 1.6 a	19.4 ± 2.5 b	38.7 ± 5.1
30 storage days			
CP30	52.3 ± 1.6 b	31.6 ± 8.7	48.7 ± 3.6
CP30-P	60.8 ± 1.5 a	29.9 ± 9.9	50.4 ± 3.0
CP40	57.7 ± 0.9 b	28.5 ± 6.2 a	44.8 ± 2.7
CP40-P	59.5 ± 1.7 a	23.0 ± 1.7 b	44.7 ± 1.0
45 storage days			
CP30	57.1 ± 6.3 b	32.2 ± 3.8	48.1 ± 3.4
CP30-P	68.8 ± 4.8 a	31.3 ± 2.6	48.2 ± 2.4
CP40	59.1 ± 0.4 b	29.4 ± 6.2	41.2 ± 2.7
CP40-P	66.3 ± 1.2 a	30.4 ± 4.2	43.8 ± 3.2

CP30: Pollinated; CP30-P: Parthenocarpic; CP40: Pollinated; CP40-P: Parthenocarpic. Values represent means ± SD (n = 5). Different letters indicate statistically significant differences among varieties of the same color, the absence of letter, no statistically different.

Antioxidant Capacity, Titratable Acidity and Total Soluble Solids

Recent studies have highlighted the antioxidant properties of *O. ficus-indica*, attributed to compounds such as polyphenols and flavonoids (Sabtain *et al*., 2021). Antioxidant activity in the pericarpel increased in all varieties during storage. No significant differences were observed in the pulp during storage; antioxidant activity ranged between 67 and 75% RSC for the four varieties, similar to those

results of Flores-Hernández *et al*. (2025) reported for the same varieties stored at 21 ± 1 °C and 61 ± 2% RH. The most significant differences were observed in the pericarpel, where yellow parthenocarpic fruits (CP40-P) showed a 66.5% increase in antioxidant activity after 45 days of storage, likely as a response to dehydration stress that induces the synthesis of antioxidant compounds (Pérez-Lamela *et al.*, 2021). Overall, parthenocarpic fruits exhibited higher antioxidant activity, although red fruits increased by only 35% between day 0 and 45 (Table 3). No positive correlation was observed between enzymatic and antioxidant activity: parthenocarpic fruits showed lower enzymatic activity but higher antioxidant activity, whereas the opposite occurred in pollinated fruits. This suggests that other non-enzymatic antioxidant systems, such as vitamins E and A, ascorbic acid, flavonoids, carotenoids, glutathione, polyphenols, allyl sulfides, curcumin, melatonin, and polyamines, might contribute to the detoxification of reactive oxygen species (ROS) independently of CAT and APX (Adiletta *et al.*, 2021).

Table 3. Antioxidant capacity (% RSC) in the pericarpel during cold storage at 10 ± 1 °C and 85 ± 2% RH in *Opuntia ficus-indica* Mill. Fruits.

Evaluation day/Variety	0 storage day	30 storage days	45 storage days
CP30	41.4 ± 10.8 b	70.8 ± 4.9 b	74.8 ± 1.1 b
CP30-P	50.7 ± 5.4 a	75.2 ± 0.3 a	77.8 ± 2.4 a
CP40	28.4 ± 0.8	82.2 ± 2.5 b	83.6 ± 0.6 b
CP40-P	39.1 ± 1.9	85.7 ± 2.4 a	86.8 ± 0.9 a

CP30: Pollinated; CP30-P: Parthenocarpic; CP40: Pollinated; CP40-P: Parthenocarpic. Values represent means ± SD (n = 5). Different letters indicate statistically significant differences among varieties of the same color.

Regarding total soluble solids content (°Brix) and titratable acidity during storage, no statistically significant differences were observed between varieties or tissues (pulp and pericarpel), remaining between 13-14 °Brix and 0.2-0.3 % acidity. These values were not affected by cold storage, as they are similar to those reported at 21 °C in postharvest conditions (Flores-Hernández *et al.*, 2024; 2025). This response can be explained because prickly pear is a non-climacteric fruit, with no marked increase in respiration or ethylene production after harvest, allowing °Brix and acidity to remain stable (Mayer-Miebach *et al.*, 2012). In addition, the high mucilage content, composed of complex polysaccharides, acts as a structural and water reserve, reducing the fruit's reliance on soluble sugars and acids as respiratory substrates, thereby contributing to the stability of these traits during storage (Liguori *et al.*, 2021).

Conclusions

Cold storage had a significant impact on weight loss and electrolyte leakage in parthenocarpic prickly pear fruits compared to pollinated fruits, the latter being more susceptible to pathogen damage. However, parthenocarpic fruits have a physiological advantage due to their greater non-enzymatic antioxidant capacity at the end of the cold storage. These results indicate that, although parthenocarpic fruits had higher dehydration predominantly in the hypanthium, further research can be focused in the use of coatings or individual packaging to mitigate water loss.

ETHICS STATEMENT
Not applicable.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

AUTHOR CONTRIBUTIONS

B.K.F.-H.: Methodology, laboratory analysis, and writing; M.L.A.-G.: Conceptualization and writing; M.L.-M.: Plant material and experimental design; A.M.-H.: Laboratory analysis; C.P.-V.: Laboratory analysis; G.C.-Z.: Discussion and writing.

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