

Article

Influence of Storage on Physiological Properties, Chemical Composition, and Bioactive Compounds on Cactus Pear Fruit (*Opuntia ficus-indica* (L.) Mill.)

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Abstract: Cactus pear (*Opuntia ficus-indica* (L.) Mill.) fruit from ‘Orito’ cultivar were stored at 2 °C and 90% RH for 28 days plus three days at 20 °C (shelf life, SL). This research analysed the changes in fruit quality parameters (weight loss, firmness, color, titratable acidity, and total soluble solids), ethylene production, respiration rate, antioxidant activity and bioactive compounds (total phenols and carotenoids) of cactus pear fruit during cold and shelf life storage. Under cold conditions, CO₂ production decreased, and ethylene production increased slightly, while under shelf life conditions CO₂ production increased and ethylene production increased more sharply. Firmness increased under cold conditions and did not change during shelf life period. The content of total soluble solids (TSS), titratable acidity (TA), pH, total carotenoids, and lipo-antioxidant activity (L-TAA) remained stable under both conservation conditions. However, hydro-antioxidant activity (H-TAA) increased under both cold and shelf life conditions, and total phenols remained stable during cold storage and increased under shelf life conditions. Besides, weight loss was acceptable under both storage conditions, and color changes were more pronounced under shelf life storage. These results show that the marketability of cactus pear fruit from ‘Orito’ cultivar was acceptable until the end of the storage under cold and shelf life conditions.

Keywords: prickly pear; storage; shelf life; fruit quality; antioxidants



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1. Introduction

Cactus pear (*Opuntia ficus-indica* (L.) Mill.) is the Cactaceae plant with the greatest economic relevance in the world [1,2]. It is a tropical or subtropical plant original from the arid and semi-arid regions of America [3], which can grow in arid and semi-arid climates [4]. Cactus pear is known by its fruit, commonly named “tunas” or “figs”. Mexico is the largest producer and consumer in the world, with the largest cultivation area [2,5]. Italy, South Africa, Chile, Israel, and Spain are also important producers [2]. In addition to the consumption of its fruit, this plant presents a wide range of applications. Some of the more important are cultivation as a forage supplement, consumption of cladodes, medical uses, non-food industrialization (for instance, the production of bioenergetics and cosmetics), and carmine production [2].

The fruit or cactus pear is generally consumed fresh, but they are highly perishable, and usually after nine days of storage at ambient temperature (19 ± 5 °C), the fruit can

show spots and rotting due to decay [6]. This fruit is classified as a non-climacteric fruit, in which cold storage reduces the respiration rate and fruit mass loss, inhibits the growth of microorganisms, and prolongs shelf life [7].

There are some studies that have evaluated the storage of cactus pear under different conditions and treatments, such as effects of storage temperature [8,9], effects of UVB light [10], and cryocauterization [6], among others. However, the success of storage depends on several factors, including the cultivar, storage atmosphere, orchard management practices (especially irrigation and mineral nutrition), and fruit maturity stage [11].

The aim of this study was to evaluate the effect of cold storage and shelf life on physical and physicochemical characteristics and bioactive compounds of fruit from a Spanish cultivar called 'Orito'. Due to the limited of studies evaluating these characteristics in cactus pear fruit, this information will be used to improve the storage of cactus pear fruit and its marketability.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Cactus pear fruit from a commercial farm (38°23'30.7" N, 0°40'13.0" W) in Orito (Alicante, Spain) were used for this study. Fruit from 'Orito' cultivar is orange and had an average weight of 125.92 ± 3.87 g. Two thousand fruit were hand-harvested in mid-August 2017 at the commercial ripening stage. The fruit was transported, under cold conditions, to the laboratory for preparation and further analyses. Once in the laboratory, the spines of fruits were removed with a brush, and 540 fruit were selected based on the absence of visual defects and by homogeneous size and color, and randomly divided into 27 lots of 20 fruit, being each a biological replicate.

Three lots were used to evaluate fruit properties at harvest. The rest of the lots were stored in a refrigeration chamber at 2 °C and 85.90% relative humidity (RH). Of these, three lots were taken at seven, 14, 21, and 28 days after harvest, in which all the analyses were carried out. The other three lots were taken and placed at 20 °C for three days to study the shelf life (SL). After each analysis, the fruit were frozen at −80 °C for total antioxidant activity (due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds), total phenolics, and total carotenoids. Quality parameters, such as weight loss, color, fruit firmness, total soluble solids (TSS), and total acidity (TA), were measured in three replicates of 20 fruit.

2.2. Ethylene Production and Respiration Rate

Ethylene production and respiration rate were measured by placing each lot in a 2 L glass jar hermetically sealed with a rubber stopper for one hour. One mL of the holder atmosphere was withdrawn with a gas syringe and used to quantify ethylene concentration into a Shimadzu TM GC-2010 gas chromatograph (Kyoto, Japan), with the characteristics explained in Díaz-Mula [12].

Another sample of 1 mL of the same atmosphere was used to quantify respiration rate by measuring CO₂ concentration into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD), with the characteristics explained in Díaz-Mula [12]. Ethylene production and respiration rate was expressed as nmol kg^{−1} s^{−1}. These analyses were made in duplicate; data are the mean ± SE of determinations made in three replicates.

2.3. Fruit Quality Parameters

Each lot of fruit were weighed using a digital balance (model BL-600; Sartorius, Madrid, Spain) to calculate weight loss. Fruit lots were weighed at day zero, and after the storage period (both cold and shelf life), weight loss was determined as the percentage of weight loss in relation to the weight at day zero. Fruit firmness was determined in each fruit as force deformation (N mm^{−1}) by using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK), which employed a force causing a

10% of deformation of the fruit diameter at day zero and 5% the rest of the days. Color, as L^* , a^* , and b^* parameters, were measured with a Minolta colorimeter CR200 model (Minolta Camera Co., Osaka, Japan) by using the CIEL*a*b* System and was expressed as Hue angle ($\tan^{-1}(b^*/a^*)$). For these parameters, data are the mean \pm standard error (SE) of individual determinations made in three replicates of five fruit.

After these non-destructive determinations, the pulp of the fruit was cut into small pieces in order to obtain a uniform sample of each replicate. A part was employed to measure total soluble solids (TSS) concentration and titratable acidity (TA), and the remaining were immediately frozen at $-80\text{ }^\circ\text{C}$ until analysis of H-TAA, L-TAA, total phenolics, and total carotenoids were made.

Total soluble solids (TSS) concentration and titratable acidity (TA) were measured in the juice of the homogeneous samples of each lot. TSS was determined in duplicate at room temperature with a digital refractometer Atago Pocket PAL-1 (Atago Co. Ltd., Tokyo, Japan) and expressed as a percentage. TA was also determined in duplicate by titration of 1 mL of juice with 0.1 N NaOH up to pH 8.1 by using an automatic titrator (TitraLab AT1000 series, Hach Tokyo, Japan), and the results were expressed as g of malic acid equivalent per kg^{-1} . Ripening index (RI) was calculated as the ratio between TSS and TA. Data are the mean \pm SE of three replicates.

2.4. Antioxidant Activity and Bioactive Compounds

Total antioxidant activity (TAA) was determined in duplicate for each lot according to the methodology of Arnao et al. [13], which allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) in the same extract. In summary, 5 g of the homogeneous sample of frozen pulp were homogenized in 15 mL of methanol:water (80:20, *v/v*) containing 1% of HCl (39%) and 2 mmol L^{-1} of NaF to inactivate polyphenol oxidase activity, and then centrifugated at $15,000\times g$ at $4\text{ }^\circ\text{C}$ for 15 min. For the quantification of L-TAA was used the upper fraction, and the lower one was used to quantify L-TAA, both made in duplicate. The reaction medium included 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), horseradish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide). Trolox ((R)-(+)-6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid) (0–20 nmol) from Sigma (Madrid, Spain) was used as a standard antioxidant to perform a calibration curve for both H-TAA and L-TAA, and results were expressed as mg Trolox equivalents kg^{-1} (fresh weight basis). Total carotenoids were quantified in the lipophilic extract [13] by reading the absorbance at 450 nm in a UNICAM Helios- α spectrophotometer (Cambridge, UK), and were expressed as mg of β -carotene equivalent kg^{-1} fresh weight, considering the $\epsilon_{\text{cm}}^{1\%} = 2560$. Total phenolics were extracted according to Tomás-Barberán et al. [14] using the same extractant described above and quantified using the Folin-Ciocalteu reagent. Briefly, 200 μL of the hydrophilic extract were diluted in the extractant described above and mixed with 2.5 mL of water-diluted Folin-Ciocalteu reagent. The mixture was incubated for 3 min at room temperature. Then, 2 mL of sodium carbonate (75 g L^{-1}) was added, and the mixture was shaken. At last, the mixture was incubated at $60\text{ }^\circ\text{C}$ for 5 min, and absorbance was measured at 760 nm. Gallic acid was used for performing a calibration curve. Results were expressed as mg gallic acid equivalent per kg fresh weight. Results were the mean \pm SE of measures made in duplicate in each of the three replicates.

2.5. Statistical Analyses

One-way analysis of variance (ANOVA) and multiple-range tests were used for sample comparisons. The method used to discriminate among the means (Multiple Range Test) was Tukey's least significant difference procedure. Significance was defined at $p \leq 0.05$. Statistical analysis was performed using XLSTAT software version 9 [15]. Figures were drawn using SigmaPlot 11.0 (Systat Software, San José, CA, USA) [16].

3. Results and Discussion

3.1. Ethylene and CO₂ Production

The fruit has been defined as climacteric and non-climacteric depending on the pattern in ethylene production and respiration rate. Ethylene is a gas of natural origin that is produced by fruit and vegetables during their metabolic processes. It is related to the growth and maturation of the fruit, inducing changes such as texture, color changes, and tissue degradation. Ethylene is considered the plant hormone responsible for the ripening process in climacteric fruits, such as tomato, apple, and melon, among others. However, non-climacteric fruit, such as pepper and grapes, present in their respiratory pattern, comparably low values of ethylene production and gradual decline production during the ripening process [12].

Classification of climacteric and non-climacteric fruit is not categorical. Some species show both patterns in different cultivars or genotypes, such as strawberry, grape, and citrus fruit [12,17]. Cactus pear fruit was classified as a non-climacteric fruit [18,19] with low respiration rates in comparison to those of other common fruit like avocado, banana, and mango [18]. However, the ‘Orito’ cultivar showed a suppressed-climacteric pattern in ethylene production and respiration rate, similar to some cultivars of plum [20,21], which showed no increase in respiration rate or in ethylene production related to ripening. Besides, respiration can be affected by the variety, the maturity stage at harvest time, the type of crop, and the environmental conditions, among others [2], and physical damage or decay cause increased respiration and ethylene production rates [18].

With respect to respiration rate in cactus pear fruit, the storage under cold conditions (2 °C) decreased the CO₂ production, changed from 231 nmol kg⁻¹ s⁻¹ at day zero to 64 nmol kg⁻¹ s⁻¹ at day seven. Then, the CO₂ production remained stable until the end of cold storage, reaching 51 nmol kg⁻¹ s⁻¹ at the end of cold storage (Figure 1A). However, when measuring shelf life conditions, CO₂ production increased slightly after 14 d (287 nmol kg⁻¹ s⁻¹) and then decreased up to values below the initials at the end of storage (182 nmol kg⁻¹ s⁻¹ at 28 d) (Figure 1A). Increasing the temperature from 2 °C to room temperature resulted in a greater increase in CO₂ production rate, but after 14 days, the production of CO₂ began to decrease under both conditions. This increase in CO₂ production in response to temperature can also be observed in other cultivars of cactus pear fruit [22] and in other aerial parts as cladodes [23]. Besides, the results obtained of the CO₂ production were in accordance with those obtained by Lakshminarayana and Estrella [19] and Corrales-García et al. [24].

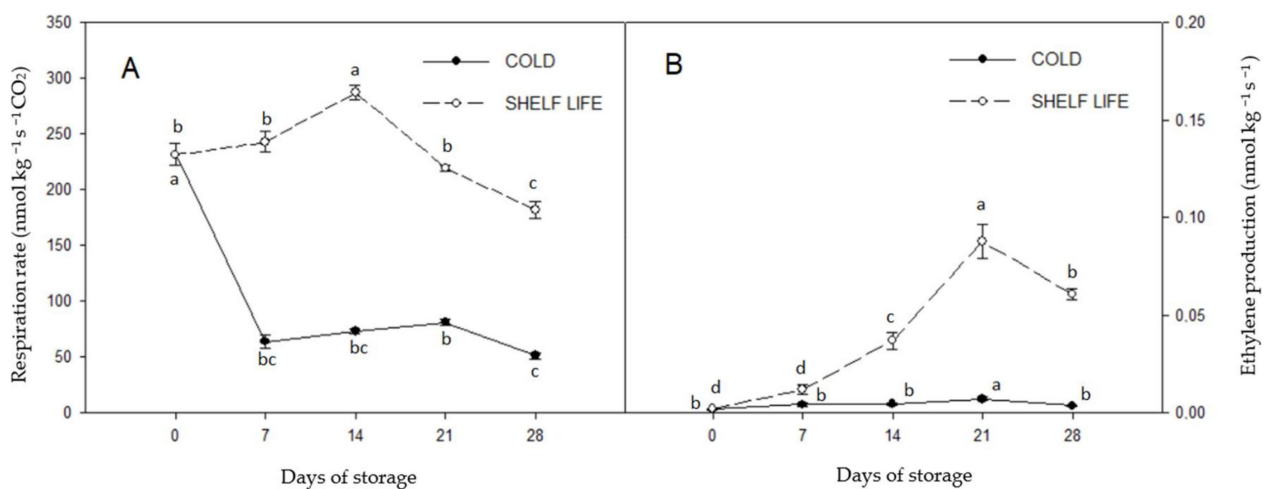


Figure 1. Changes in respiration rate (CO₂) (A) and ethylene concentration (B) in cactus pear fruit during storage under cold and shelf life conditions. Data are the mean ± standard error (SE) ($n = 6$). Tukey’s test result at a 95% confidence level is shown. Different letters indicate significant differences ($p < 0.05$) during each storage time.

Regarding ethylene production, during cold storage, this compound was slightly increased until day 21 from $0.002 \text{ nmol kg}^{-1} \text{ s}^{-1}$ to $0.007 \text{ nmol kg}^{-1} \text{ s}^{-1}$ and decreased to $0.003 \text{ nmol kg}^{-1} \text{ s}^{-1}$ at the end of storage (Figure 1B). Shelf life storage showed the same trend, but the increase was higher, and at day 21, the ethylene production reached $0.09 \text{ nmol kg}^{-1} \text{ s}^{-1}$ and $0.06 \text{ nmol kg}^{-1} \text{ s}^{-1}$ at the end of storage (Figure 1B). These low ethylene emission rates showed that the cactus pear presented with a suppressed-climacteric pattern in ethylene production, and its metabolism decreased at low temperatures. The results of this study were in accordance with others [22,25], which showed that the ethylene production of cactus pear fruit was low under cold conditions, but the increase in temperature to 20°C caused an increase in ethylene production up to ten times higher.

3.2. Fruit Quality Parameters

The rate of postharvest water loss in fruits depends primarily on the external vapor pressure deficit, although it can be influenced by other factors such as the intrinsic and extrinsic characteristics of the fruit. Fruit with thick peels, such as citrus fruit, bananas, or cactus pear, can lose a significant quantity of skin moisture without affecting edible quality [12].

In this study, cactus pear fruit showed a low weight loss during the 28 days of storage, both in cold and shelf life conditions. The weight losses at the end of storage were $2.22 \pm 0.08\%$ in cold storage and $3.71\% \pm 0.40$ after the shelf life period. Under shelf life conditions, weight loss increased significantly past day 21. However, under shelf life conditions, weight loss was higher between days seven and 21 (Table 1). According to Lamúa [26], in most vegetable species, weight losses above 6–8% cause an irreversible alteration of sensory quality, affecting its commercial quality. Because the weight losses in this study did not reach 4%, cactus pear fruit from the ‘Orito’ cultivar maintained their quality and marketability. These weight losses under cold conditions were similar in ‘Cristalina’ and ‘Alfajayucan’ cultivars and lower than other cultivars studied by López-Castañeda et al. [27]. The ‘Copena-Torreaja’ cultivar showed more than 10% weight loss when exposed four days at room temperature conditions after cold storage, but the ‘Cristalina’, ‘Picochulo’ and ‘Burróna’ cultivars showed a weight loss of less than 4% under the same conditions, which agree with the results of this study [24]. ‘Giallia’ cultivar showed 4.1% of weight loss after seven weeks of storage at 6°C , and 5.7% after seven weeks at 6°C and three days of a simulated marketing period (shelf life) [22].

Table 1. Fruit quality parameters (total soluble solids (TSS), total acidity (TA), ripening index and weight loss) calculated in cactus pear fruit during conservation under cold and shelf life (SL) conditions. The values represented are the mean.

Days of Storage	0	7	14	21	28	7 + SL	14 + SL	21 + SL	28 + SL
TSS (%)	14.9	14.8	14.5	14.3	14.5	14.1	14.5	14.4	14.0
TA (g malic acid kg^{-1})	0.9	0.8	0.8	0.9	0.9	0.8	0.9	0.9	0.9
Ripening index	168 b	185 a	180 a	160 b	161 b	176 a	161 b	160 b	155 b
Weight loss (%)	0 g	0.28 f	1.05 e	1.97 c,d	2.22 b,c	1.13 e	1.82 d	2.43 b	3.71 a

The different letters within the rows indicate significant differences according to the Tukey’s test ($p < 0.05$).

Changes in fruit texture during postharvest storage are due to dehydration and changes in the components of the middle lamella and primary cell wall, which causes fruit softening. These processes depend on the class of fruit and even of the cultivar [12]. Cold storage of cactus pear fruit from ‘Orito’ cultivar increased firmness from day seven, reached values of $11,990 \text{ N m}^{-1}$. However, no significant differences were found under shelf life conditions, in which firmness showed values between $9.17\text{--}10.1 \text{ N mm}^{-1}$ (Figure 2). At the end of storage, firmness increased 16.6% under cold conditions and decreased 3.58% under shelf life conditions with respect to day zero. No visual chilling injuries were detected during cold storage of ‘Orito’ fruit (data not shown). Excess of fruit softening limited shelf life, storage, because could increase the physical damage during management and make fruit more susceptible to pest and diseases. In this sense, cactus pear fruit from the ‘Orito’

cultivar showed an acceptable quality and marketability because the loss of firmness did not occur during cold storage, but rather its increase and firmness loss during shelf life conditions was very low compared to that other fruit such as tomato (55%), apricot (72%), or lemon (26%) under similar conditions [28]. The results of this study are in accordance with other authors [22,25] who obtained that cold storage prevented firmness loss in cactus pear fruit, and this rapidly declined when fruit was kept at 20 °C.

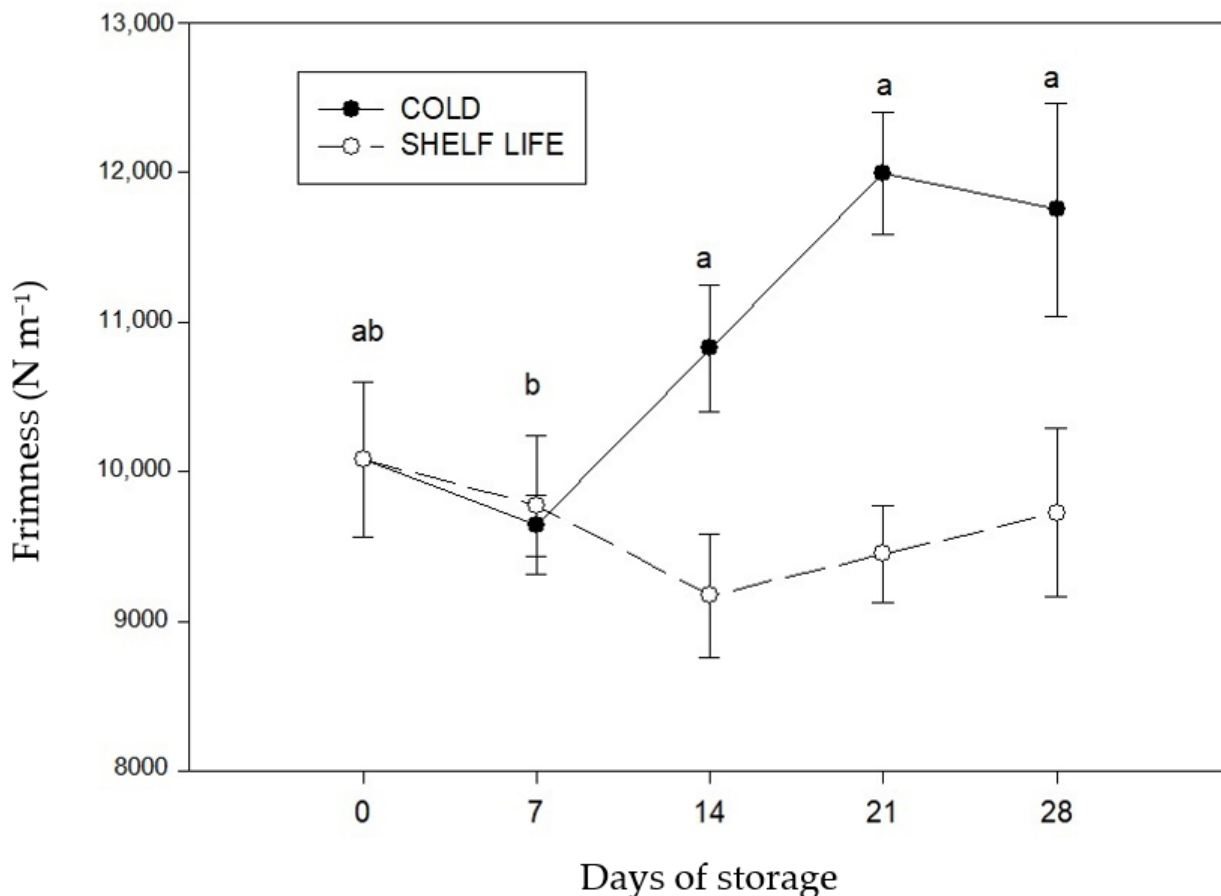


Figure 2. Changes in respiration rate (CO₂) (a) and ethylene concentration (b) in cactus pear fruit during storage under cold and shelf life conditions. Data are the mean ± SE (*n* = 6). Tukey's test at a 95% confidence level is shown. Different letters indicate significant differences (*p* < 0.05) during each storage time.

Levels of sugars are an important factor in determining the taste of ripe flesh fruit. In cactus pear fruit, the main sugar is glucose, followed by fructose, with levels at harvest in a range of 103–144 g L⁻¹ of glucose and 57–88 g L⁻¹ of fructose [29]. The measure of total soluble solids (TSS) is important to estimate the sugar content in fruit and to determine its degree of sweetness and thus estimate consumer acceptance, along with volatile compounds, which were studied in 'Orito' cultivar showed mainly green and fatty notes [30]. However, the perception of taste by consumers is not only linked to these parameters, and TA is also an important factor. Thus, the ripening index (TSS/TA) is used to estimate the degree of fruit acceptance [12]. In this study, the values of TSS and TA remained stable during both shelf life and cold conditions because cactus pear, in this parameters, showed a non-climacteric fruit pattern, in which the concentration of nutrients remains in the fruit without substantial changes during storage [2], while in climacteric fruit such as kiwifruit or nectarine, the content of TSS increased and TA decreased during postharvest, although these changes are considerably dependent of the fruit species and cultivars [12,28]. The 'Orito' cultivar showed TSS content between 14% and 14.9% (Table 1),

similar to the results obtained by Andreu et al. [31]. Values of TSS of >12–13% are required to ensure that the fruit has good quality, so the TSS content of the 'Orito' cultivar was appropriate [3]. TA showed values close to $0.9 \text{ g} \times \text{kg}^{-1}$ (Table 1), the same as those obtained by Schirra et al. [22] in the 'Giulla' cultivar and slightly higher than those obtained by Graça-Miguel et al. [32] in the 'Orange', 'Green', and 'Rossa' cultivars. This caused the ripening index (RI) in cactus pear fruit to remain at about 155–185 (Table 1).

Colored fruit has always been part of the human diet and helps us to identify food and evaluate its palatability. In addition to defining the aesthetic value of fruit, color predetermines consumers' expectations of flavor and taste, modulates appetite, and is a major issue for the food industry. However, color may be altered during fruit storage through the action of light, temperature, and oxygen, among others. The CIEL*a*b* System (International Commission on Illumination, Vienna) has been adopted by the food industry for measuring the color of products and color changes during storage [28]. The L* parameter, which reflects color luminosity, did not show significant differences during cold storage but did during shelf life conditions, decreased from an initial value of 57.8 ± 0.40 to 54.2 ± 0.82 after 28 days (data not shown). Regarding the Hue angle, there were no significant differences in this parameter under cold conditions. However, under shelf life conditions, the Hue angle decreased after seven days and stayed constant until the rest of storage, from an initial value of 75.2 ± 0.08 to 67.7 ± 1.59 after seven days (data not shown). Decreases in the Hue angle are related to peel darkening in fruit. The trend of these parameters was in accordance with that obtained by other authors [8,33], who analyzed changes in the color of *Opuntia ficus-indica* and *O. albiarpa* fruit under cold and shelf life conditions.

3.3. Bioactive Compounds and Antioxidant Activity

There is ample evidence about the health benefits of cactus pear fruit consumption, mainly due to its antioxidant activity [31,34,35]. Phenolic compounds are a group of secondary natural metabolites in plants that represent the strongest antioxidants in plant foods [28]. During the cold storage of cactus pear fruit, total phenol content remained stable, with values between 640–810 mg kg^{-1} (Figure 3). These results were in accordance with those obtained by Coria-Cayupán et al. [36] in the fruit of the 'Yellow' cultivar of *Opuntia megacantha*. However, during the shelf life period increased after seven days (903 mg kg^{-1}) and decreased at the end of storage (690 mg kg^{-1}) (Figure 3). The concentration of these compounds was in accordance with those obtained by Moussa-Ayoub et al. [37] but was lower than those obtained by Ramírez-Ramos et al. [38]. The variation of the content of phenolic compounds may be due to various factors, such as agronomic practices, environmental conditions, the pre- and postharvest management of fruit, and the reduction of these compounds during fruit ripening [38]. Anorve-Morga et al. [39] analyzed changes in phenolic compounds under different storage temperatures in cactus pear fruit and concluded that during storage, there was an increase in phenol content, which was directly influenced by temperature, which could explain the results of these study.

The antioxidant capacity of fruit can be carried out separately on hydrophilic and lipophilic extracts to evaluate if antioxidant activity is derived from water-soluble (H-TAA) or lipo-soluble (L-TAA) molecules [28]. Both cold and shelf life storage increased H-TAA, reached the maximum concentration after 21 days in both storage conditions (Figure 4A). This trend had been reported in non-climacteric fruit such as citrus and plum fruit under cold storage [40,41]. However, this behavior was the opposite in jujube fruit, a climacteric fruit, whose H-TAA decreased significantly with respect to day zero under cold conditions [42]. By contrast, L-TAA in cactus pear fruit, which was significantly lower than H-TAA, remained stable during cold and shelf life storage periods, showed values around 90 mg kg^{-1} (Figure 4B). These results suggested that hydrophilic compounds contributed more than lipophilic compounds in the antioxidant capacity of cactus pear fruit.

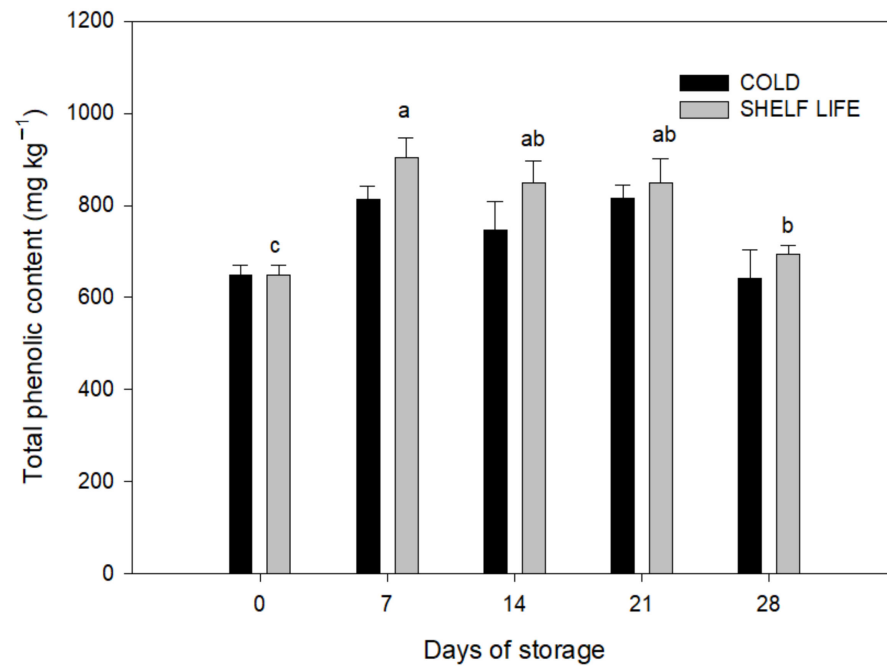


Figure 3. Total phenolic content changes in cactus pear fruit during cold and shelf life storage. Data are the mean \pm SE ($n = 6$). Tukey's test at a 95% confidence level is shown. Different letters indicate significant differences ($p < 0.05$) during each storage time.

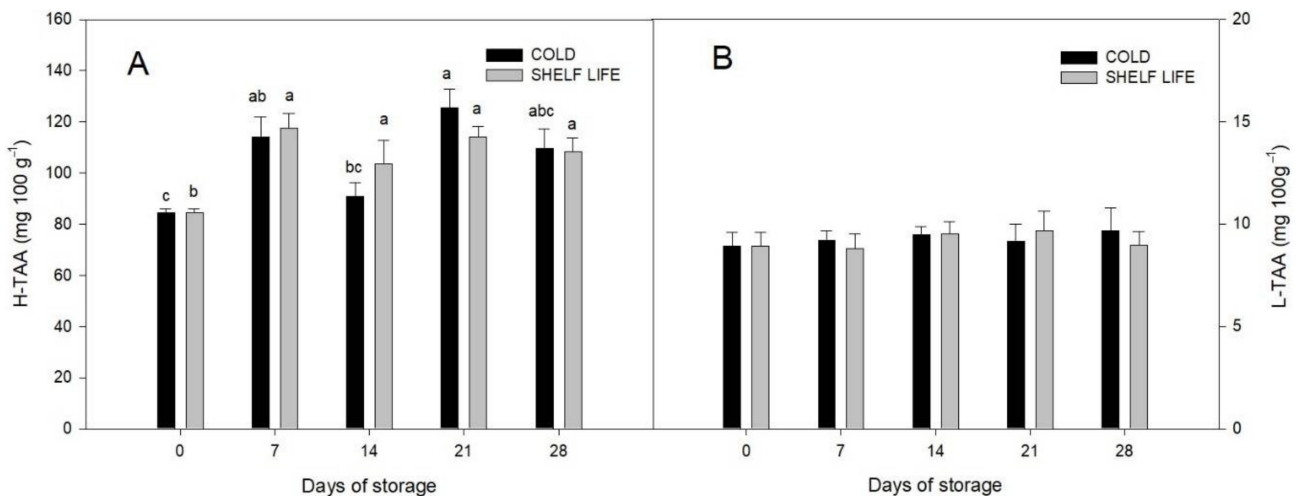


Figure 4. Changes in H-TAA (A) and L-TAA (B) during storage under cold and shelf life conditions of cactus pear fruit. Data are the mean \pm SE ($n = 6$). Tukey test's at a 95% confidence level is shown. Different letters (a,b,c) indicate significant differences ($p < 0.05$) during each storage time.

Carotenoids are lipophilic compounds that are responsible for most yellow to red color of fruit and present antioxidant properties. Cactus pear fruit showed a very low concentration of these compounds (1.20 mg kg^{-1} on average, data not shown). These compounds showed a similar trend to L-TAA, without changes during storage under cold and shelf life conditions. The concentration of carotenoids in this study was lower than those obtained by Kuti [43] in a green-skinned cactus pear cultivar. Oranges, which are non-climacteric fruit, did not show changes in carotenoid concentration during cold storage, similar to the behavior of 'Orito' fruit [44].

4. Conclusions

Based on the results of this study, the ‘Orito’ cultivar showed a suppressed-climacteric fruit profile because of its ethylene production and respiration rate during storage. The storage under cold conditions (2 °C, 85–90% HR) maintained fruit quality parameters in optimal values for up to 28 days. Besides, fruit quality parameters were acceptable during shelf life storage; however, cold conditions were more appropriate. These results showed that the marketability of cactus pear fruit from the ‘Orito’ cultivar would be possible up to 28 days after harvesting. Thus, further investigation is required to evaluate how long it is possible to preserve the marketability of this fruit and experiment with other conditions, such as modified atmosphere packaging.

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