Postharvest Biology and Technology 150 (2019) 105–111

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Storage alters physicochemical characteristics, bioactive compounds and antioxidant capacity of cactus pear fruit

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ARTICLE INFO

Keywords:
Opuntia spp.
Phenolic compounds
Antioxidant activity
Betalains

ABSTRACT

Fruit of *Opuntia* spp. are flavorful with high bioactive composition, but the effects of pre-marketing storage time and conditions on the primary functional properties of the fruit have been not explored. The objective of this study was to evaluate the effects of storage temperature over time on physicochemical characteristics and bioactive components of fruit from two pigmented cactus pear cultivars. Fruit from each cultivar were assessed at harvest (H) and after storage at room temperature (RT; 24 °C ± 1 °C and 37 ± 8% relative humidity (RH) for 35 d), or in a cold room (10 °C and 95% RH) for 77 d for 'Amarilla Olorosa' fruit or 112 d for 'Roja Lisa' fruit. Fruit mass loss (FML) was calculated and juice from each fruit was used to determine of total phenolic content (TPC), phenolic acids contents (PA; gallic, protocatechuic, benzoic, and hydroxybenzoic), antioxidant activity, betalains and vitamin C. 'Roja Lisa' fruit had the least FML under both storage conditions. TPC, PA, betalains, vitamin C, and antioxidant capacity were highest in fruit stored in cold stored fruit of both cultivars. In contrast, dehydroascorbic acid was detected only in 'Roja Lisa' fruit at H or in cold storage. Our results suggest that cactus pear fruit stored at RT or at cold storage for 5 weeks, or more than 11 weeks, respectively, with maintenance and enhancement of some nutraceutical properties.

1. Introduction

The most commonly known fruit of *Opuntia* is cactus pear, a thin-skinned fruit with a juicy, and sweet pulp (Barbera et al., 1992). It is a native species of Mexico that has spread around the world, including Africa, Australia, and the Mediterranean basin, and is presently cultivated in Israel, Italy, Morocco, Tunisia, Algeria, South Africa, the United States, Argentina, and Chile (Sáenz et al., 2013). Cactus pear has gained attention over the past two decade for its nutritional and nutraceutical or functional characteristics (Piga, 2004). Cactus pear fruit is a good source of minerals, protein, dietary fiber, and phytochemicals such as β-caroteno and betalains (Feugang et al., 2006; Bensadón et al., 2010), vitamin C (Albano et al., 2015), lipid soluble antioxidants (Tesoriere et al., 2005) and diverse bioactive compounds such as proanthocyanindis, hydrolyzable tannins (Bensadón et al., 2010), and betalains (Butera et al., 2002; Castellar et al., 2006; Cejudo-Bastante et al., 2014; Albano et al., 2015). Cactus pear fruit also contains various phenolics: quercetin, myricetin, kaempferol, and luteolin, among others (Fernández-López et al., 2010; Cejudo-Bastante et al., 2014). Outstanding antioxidant capacity, a protective effect against tetrachloride-induced injury (Galati et al., 2005), anti-ulcerogenic activity (Galati et al., 2003), and cancer cell cytotoxicity have been reported (Chavez-Santoscoy et al., 2009).

Cold storage reduces the respiration rate and fruit mass loss (FML) of most climacteric fruit, inhibits growth of microorganisms and extends shelf life (Crisosto and Mitchell, 2002). Early studies on cactus pear fruit (non-climacteric fruit) conservation revealed that the primary postharvest problems are loss of physicochemical characteristics and dehydration (Berger et al., 1978). Techniques investigated as methods to extend fruit shelf life include application of fungicides, natural waxes or edible plastic films, hot water treatments, and cold storage (Schirra et al., 1996, 1997a; Schirra et al., 1997b, 2000; Fallik, 2004; Berger et al., 2002). Depending on the cultivar, cactus pear fruit are susceptible to chilling injury when stored at 6 °C or 10 °C and are susceptible to FML (Schirra et al., 1996; Varela-Gámez et al., 2014). One study suggested an ideal storage temperature for cactus pear fruit of 10 °C, which provided conservation for up to 60 d (Martínez-Soto et al., 1999). However, success of this storage regime depended on the

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https://doi.org/10.1016/j.postharvbio.2019.01.001
Received 24 August 2018; Received in revised form 1 January 2019; Accepted 1 January 2019

cultivar and storage atmosphere (Zegbe et al., 2015), and conditions during shipping of fruit to its final destination. Transport and marketing are an important part of the value chain for delivering high-quality cactus pear fruit to the customer. Transport normally takes 3 d to 5 d in trucks at 10 °C to the closest wholesale markets and it can take up to 30 d from production zones to distant markets (Sáenz et al., 2013). Information on the effects of such storage on cactus pear physicochemical characteristics is limited. In addition, no studies have examined the effect of cold storage on bioactive compounds or antioxidant capacity of cactus pear fruit.

The objective of this study was to evaluate the effect of storage temperature over time on physicochemical characteristics and bioactive components of fruit from two pigmented cactus pear cultivars. We selected the cultivars 'Roja Lisa' (red-pulped) and 'Amarilla Olorosa' (yellow-pulped) because of their high desirability in local and export markets.

2. Materials and methods

2.1. Experimental site and plant material

The experiment was set up at the Campo Experimental Zacatecas, Calera, Zacatecas, México (22°54′31″N; 102°39′34″W; elevation 2 197 m). The experimental station receives 416 mm annual rainfall with 75% occurring between July and October and has an annual mean temperature of 14.6 °C. Average annual pan evaporation is 1 609 mm. The orchard soil has a loam texture, 1.73% organic matter content at pH 7.75. Eight year old cactus pear cultivars (*Opuntia ficus-indica* L. Mill cv. 'Roja Lisa' and *Opuntia* spp. cv. 'Amarilla Olorosa') were used. 'Roja Lisa' is red-pulped and early-maturing and 'Amarilla Olorosa' is yellow-pulped and late-maturing. Plants were spaced at 4 m between rows and 3 m within the row and trained to an open vase system. The cultural practices in the cactus pear orchard included drip irrigation, cladode pruning, fruit thinning, row fertigation, and pest and weed control as required.

2.2. Experimental design

Fruit from selected plants of 'Roja Lisa' and 'Amarilla Olorosa' was harvested randomly on July 14 and on August 19, 2014, respectively, at the mature green stage from different cladodes and transported to the laboratory, where they were numbered and the spines and glochids were removed. The fruit was then treated with a solution of 1% chlorine and 2.5 mL L^{-1} copper sulfate. Three sets of 72 fruit each were randomly allocated per cultivar. The first was used for chemical analysis at harvest. The second set was stored at room temperature [24 °C ± 1 °C and 37 ± 8% ambient humidity (RH) for 35 d]; these storage conditions mimic those used by commercial growers. The third fruit set was stored in a cold room (10 °C and 95% RH) for 77 d for 'Amarilla Olorosa' and 112 d for 'Roja Lisa'.

2.3. Fruit mass loss and juice extraction

For each storage condition, each individual fruit was weighed weekly with a digital scale (VE-303, Velab, USA) until they reached, on average, 8% fruit mass loss (FML). FML was calculated as the percentage reduction from initial weight. After that, juice from each fruit was extracted, separated from the seeds, and stored at - 80 °C for further analysis.

2.4. Phenolic compounds

Cactus pear juice was analyzed as described to determine total soluble phenols (TSP; Wolfe et al., 2003), total flavonoids (TF; Ying and Wan, 2012), and condensed tannins (CT; Deshpande and Cheryan, 1985). Simple phenols were identified and quantified by high performance liquid chromatography (RP-HPLC-DAD) using a HP 1200 series HPLC quipped with a diode array detector. Phenolic acids, flavonoids and flavanones were identified by their retention time and DAD comparison with spectra from authentic standards. To improve accuracy, the peaks of simple phenols were compared to samples spiked with standards of the suspected compounds (SIGMA). Rutin, quercetin and gallic, 3,4-hydroxybenzoic, 4-hydroxybenzoic, vanillic and syringic acids were detected at 260 nm; (+)-catechin, epicatechin (EC), (-)-epigallocatechin gallate (EGCg), naringerin, naringin, eriocitin, hesperidin and protocatechuic, benzoic, hydroxibenzoic, and ellagic acids were detected at 280 nm.

2.5. Antioxidant activity

A 7 mM solution of the radical cation ABTS•+ was reacted with a 2.45 mM potassium persulphate solution in the dark at room temperature for 12 h to16 h before use. Then, the radical cation was diluted with potassium phosphate-buffered saline solution to an absorbance of 0.700 nm ± 0.020 at 734 nm. To determine the Trolox equivalent antioxidant capacity (TEAC), 10 µL sample was mixed with 990 L of the radical solution. Absorbance was monitored at 734 nm for 6 min with a spectrophotometer (JeanWay 6305, Staffordshire, United Kingdom); the decrease in absorption after 6 min was used to calculate the TEAC value as described by Re et al. (1999) and Esparza-Martínez et al. (2016). TEAC was reported as mmol Trolox equivalent per kg on a fresh weight basis.

Fluorescein (FL) was used as a probe to determine the oxygen radical absorbance capacity (ORAC). The reaction was started by thermal decomposition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) at 37 °C in 75 mM phosphate buffer pH 7.0 (PBS). A stock solution of 44 mg FL in 100 mL PBS was prepared and stored at 5 °C in the dark until used. A working solution was prepared daily by diluting 0.167 mL stock solution in 25 mL PBS. The APPH solution was prepared daily by making 600 mg APPH up to 10 mL with PBS. A Trolox solution (20 µM in PBS) was used as a reference standard and prepared daily and stored at -20 °C until used. Given the sensitivity of the ORAC assay, samples must be diluted before analysis to avoid interference with color. 50 µL FL and 50 µL sample, blank, or 20 µM Trolox standard were added to 25 µL 221 mM AAPH and the fluorescence was determined every 5 min until the relative fluorescence intensity was < 5% of the value of the initial reading. The ORAC value was expressed as µM Trolox equivalents by comparison with a Trolox standard curve as described (Ou et al., 2001). A fluorometer counter (PerkinElmer FL 6500, Waltham, USA) was used with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Cactus juice (20 µL) was diluted appropriately in dimethyl sulfoxide (DMSO), then mixed with 180 µL 1,1-diphenyl-2-picrylhydrazyl (DPPH; 40 µg in methanol) for the radical scavenging assay as described (Clarke et al., 2013). Samples were placed in wells of a 96-well plate and kept in the dark for 15 min, then absorbance was measured at 540 nm using a Multiskan Ascent plate-reader (Thermo Electron Corporation, Mexico City, Mexico). DMSO and quercetin solution in DMSO were used as blanks and run simultaneously with samples. DPPH antioxidant capacity was reported as mmol Trolox equivalents per kg on a fresh weight basis.

For the ferric reducing ability of plasma (FRAP) assay, 900 µL of the FRAP reagent, containing 2,4,6-tripydryl-S-triazine (TPTZ), $FeCl_3$ and acetate buffer, were mixed with 90 µL of distilled water and 30 µL of sample juice (Benzie and Strain, 1996; Pulido et al., 2000). The absorbance values at 595 nm were taken every 15 s at 37 °C, using a spectrophotometer (JeanWay 6305, Staffordshire, United Kingdom). Readings were taken at 30 min, given that at 4 min, the time that is usually taken, the reaction to form the Fe-TPTZ complex has not yet finished (Pulido et al., 2000).

2.6. Betalains

Betacyanin and betaxanthin fractions were determined spectrophotometrically by diluting 1 mL cactus juice 1:10 with water acidified to pH 3 with trifluoroacetic acid as described (Stintzing et al., 2002). Betalains from diluted cactus juice were separated on a C18 cartridge (ABC Instrumentation, Mexico, D.F.), previously activated with three volumes 100% methanol and then washed and desalted with acidified water (pH 3). The betaxanthin fraction (BXF) was eluted with 100% methanol and the betacyanin fraction (BCF), with acidified methanol (methanol/acidified water pH 2). Betalains were determined using the Nilsson equations: betacyanins (%) = ((a/1129) × DF × 100), betaxanthins (%) = ((y/750) × DF × 100); where a = 1.095(A_{538}-A_{600}), y = A_{476} - (A_{538} - a) - (a/3.1), where A is the absorbance and DF is the dilution factor (Nilsson, 1970).

2.7. Vitamin C

Ascorbic and dehydroascorbic acids were determined as vitamin C in a HP 1100 series, RP-HPLC-DAD (Agilent ChemStation Software plus, Santa Clara, CA, United Sataes) by using a Zorbaxoctadecylsilane (ODS-C18) reverse-phase column. Centrimide (5 mM) and KH_2PO_4 [50 mM in methanol/water [1:99 (v/v), pH 4.6] was the mobile phase. Ascorbic and dehydroascorbic acids were monitored at 261 and 348, respectively (Corrales-Aguayo et al., 2008).

2.8. Statistical analysis

The data were analyzed using a completely random model using JMP.5.0.1 software (A Business Unit of SAS, 1989–2003, SAS Institute, Cary, NC, USA). Treatment means were separated by the minimum significant difference of the Tukey's test at $p \leq 0.05$.

3. Results and discussion

3.1. Fruit mass loss (FML)

After harvest, transpiration is the cause of FML in cactus pear fruit (Cantwell, 1995; Corrales-García et al., 2006). As for other fruit, FML at or above 8% results in a wilted or shriveled appearance among other perishable issues (Cantwell, 1995). Under room temperature (RT) or cold room (CR) storage conditions, there was less FML in 'Roja Lisa' than in 'Amarilla Olorosa' fruit (Fig. 1). However, 'Roja Lisa' had greater storage potential than 'Amarilla Olorosa', because the FML threshold for observing shrivel symptoms was established at ≈ 8% (Cantwell, 1995), which did not occur in 'Roja Lisa' during this study. Contrary to other studies (Martínez-Soto et al., 1999), fruit from both cultivars could be stored at CR (10 °C and 95% RH) for over two months (Zegbe et al., 2015). The fruit used in this experiment was collected from an irrigated orchard and irrigation may induce favorable changes to the fruit epidermis that minimize FML and favor both longer storage and shelf life (Zegbe and Serna-Pérez, 2018).

Cactus pear fruit are susceptible to chilling injury (CI) and decay. However, the incidence of these disorders depends on orchard management, storage conditions, and fruit maturity stage (Schirra et al., 1999a, 1999b). In this experiment, 2.7% of 'Roja Lisa' fruit and 15.3% of 'Amarilla Olorosa' fruit developed decay during CR storage. These percentages were less than those found in cactus pear fruit of 'Gialla' stored under CR conditions either 45 d (Schirra et al., 1999a) or 49 d (Schirra et al., 1999b), or in other fruit clones of *O. ficus-indica* stored at CR conditions for 21 d (Rodriguez et al., 2005). The tolerance of 'Amarilla Olorosa' and particularly 'Roja Lisa' to decay could be associated with genotype, maturity, or postharvest handling. Our fruit were treated with chlorine and copper sulfate after spines and glochids were removed before storage in CR. These factors could contribute to extend fruit storage and minimize decay in both cultivars.

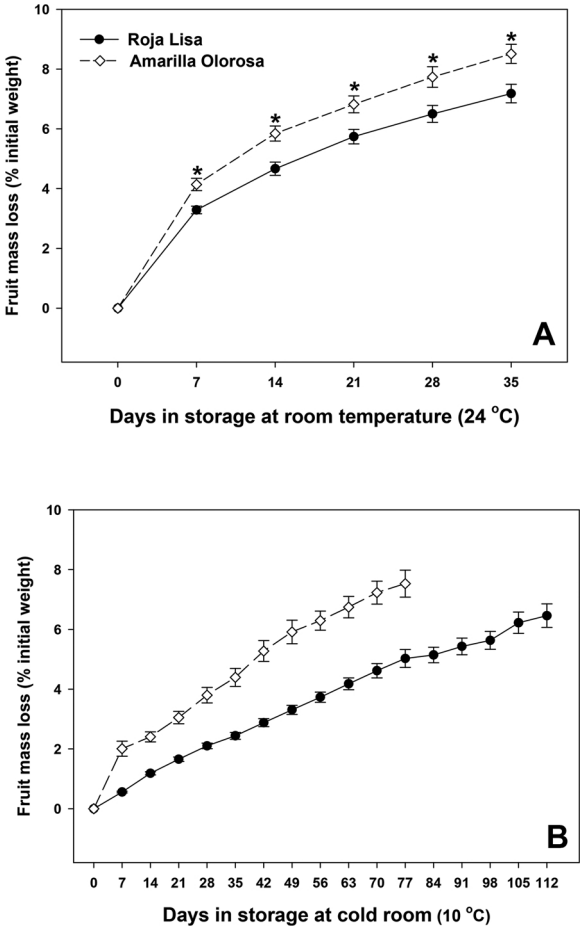

Fig. 1. Cumulative fruit mass loss for 'Roja Lisa' and 'Amarilla Olorosa' cactus pear fruit during room temperature (A) or cold room (B) storage. At each sample date, vertical bars indicate confidence intervals and the asterisks represent significant differences both at $p \leq 0.05$.

Table 1
Total soluble phenolics (TSP), total flavonoids (TF), and condensed tannins (CT) in 'Roja Lisa' and 'Amarilla Olorosa' cactus pears at harvest and stored under room temperature (RT) and cold room (CR) conditions. Means ± standard deviation (n = 72). Means in the same column with different letters indicate significant differences by Tukey's test at $p \leq 0.05$.

Assessment condition/ cultivar	TSP (mg kg^{-1})	TF (mg kg^{-1})	CT (mg kg^{-1})
At harvest			
'Roja Lisa'	97 ± 1 d	31 ± 1 f	84 ± 3 b
'Amarilla Olorosa'	94 ± 2 e	47 ± 5 d	103 ± 4 a
Stored at			
RT (24 °C and 37 % RH for 35 d)			
'Roja Lisa'	143 ± 1 c	43 ± 2 e	42 ± 3 f
'Amarilla Olorosa'	140 ± 1 c	56 ± 2 c	60 ± 4 d
CR (10 °C and 95 % RH for 77 d for 'Amarilla Olorosa' and for 112 d for 'Roja Lisa')			
'Roja Lisa'	176 ± 1 a	70 ± 2 a	57 ± 9 e
'Amarilla Olorosa'	156 ± 5 b	67 ± 2 b	68 ± 5 c

3.2. Phenolic compounds

Total soluble phenols (TSP), total flavonoids (TF), and condensed tannins (CT) in fruit from both cactus pears cultivars were affected by storage length and temperature (Table 1). The TSP in 'Roja Lisa' fruit increased by 47% or 81% and the TF by 39% or 126% under RT or CR conditions, respectively, over their values at harvest. Similar results were observed in 'Amarilla Olorosa' fruit. In contrast, CT decreased in

both cultivars at both storage temperatures. The increased TSP and TF could be, in part, due to FML. However, the storage period and conditions may have increased these two compounds. The low vapor pressure deficit (VPD) associated with a long storage period may enhanced TSP and TF concentrations in CR fruit more than high VPD in RT fruit. Fruit stored at RT (20 °C + 60 °C or 70% RH) showed the same pattern in pigmented and non-pigmented cactus pear cultivars (Ramírez-Ramos et al., 2015). The TSP concentrations of our fruit at harvest were similar to the 22.3 mg kg^{-1} GAE to 195.5 mg kg^{-1} GAE reported by one study (Chavez-Santoscoy et al., 2009), and lower than the 698.0 mg kg^{-1} GAE to 892.0 mg kg^{-1} GAE reported in another study (Albano et al., 2015). These differences could be explained by the use of different cultivars and maturity stages.

Condensed tannins are associated with antioxidant capacity, fruit color, and astringency (Chung et al., 1998; Koleckar et al., 2008). Fruit from both cactus pear were harvested at the green-mature stage; therefore, the decrease under CR conditions may be associated with reduced astringency rather than a loss of antioxidant activity. Flavonoids display a remarkable array of pharmacological action, which suggest that they may affect the function of inflammatory cells and prevent heart disease and cancer (Middleton et al., 2000).

3.3. Phenolic acids

Four out of 28 phenolic acids with available commercial standards were detected and quantified for the first time in cactus pears fruit: gallic, protocatechuic, benzoic, and hydroxybenzoic acids (Fig. 2; Table 2). In 'Roja Lisa' fruit, gallic acid increased from harvest during CR storage. Relative to fruit at harvest, gallic acid increased by 49% and 97% in RT and CR fruit, respectively. Although the total concentration of gallic acid in 'Amarilla Olorosa' fruit was less than in 'Roja Lisa' fruit under all conditions, its concentration increased, respectively, by 53% and 185% in RT fruit or CR fruit over those at harvest, respectively. Similar results were observed for the other three phenolic acids detected, except for benzoic acid, which was not detected in stored 'Amarilla Olorosa' fruit. Benzoic acid is a natural preservative in foods (Fujiyoshi et al., 2018). The low concentration or absence of benzoic acid in 'Amarilla Olorosa' fruit possibly contributed to its shorter storage at RT. In contrast, gallic acid and its catechin derivatives have several biological and pharmacological activities, including antioxidant and apoptosis (Ow and Stupans, 2003; Lu et al., 2006).

Table 2
Phenolic acid concentrations (mg kg^{-1}) in 'Roja Lisa' and 'Amarilla Olorosa' cactus pears at harvest and stored under room temperature (RT) and cold room (CR) conditions. Means ± standard deviation (n = 72). Values not detected are expressed as ND. Means in the same column with different letters indicate significant differences by Tukey's test at $p \leq 0.05$.

Assessment condition/cultivar	Gallic	Protocatechuic	Benzoic	Hydroxy-benzoic
At harvest				
'Roja Lisa'	67 ± 3 d	338 ± 4 ab	200 ± 8 c	475 ± 4 c
'Amarilla Olorosa'	34 ± 4 f	82 ± 2 c	11 ± 6 d	105 ± 9 d
Stored at RT (24 °C and 37 % RH for 35 d)				
'Roja Lisa'	100 ± 2 b	313 ± 82 ab	271 ± 29 b	648 ± 53 b
'Amarilla Olorosa'	52 ± 4 e	91 ± 69 c	ND	150 ± 36 d
CR (10 °C and 95 % RH for 77 d for 'Amarilla Olorosa' and for 112 d for 'Roja Lisa')				
'Roja Lisa'	132 ± 6 a	466 ± 97 a	497 ± 60 a	1062 ± 32 a
'Amarilla Olorosa'	97 ± 9 c	259 ± 53 b	ND	161 ± 66 d

3.4. Betalains and vitamin C

Betalain concentrations in cactus pear juice are related to fruit color, and so is much greater in red-pulped cultivars as reported previously (Ramírez-Ramos et al., 2015; Santos-Díaz et al., 2017) (Table 3).

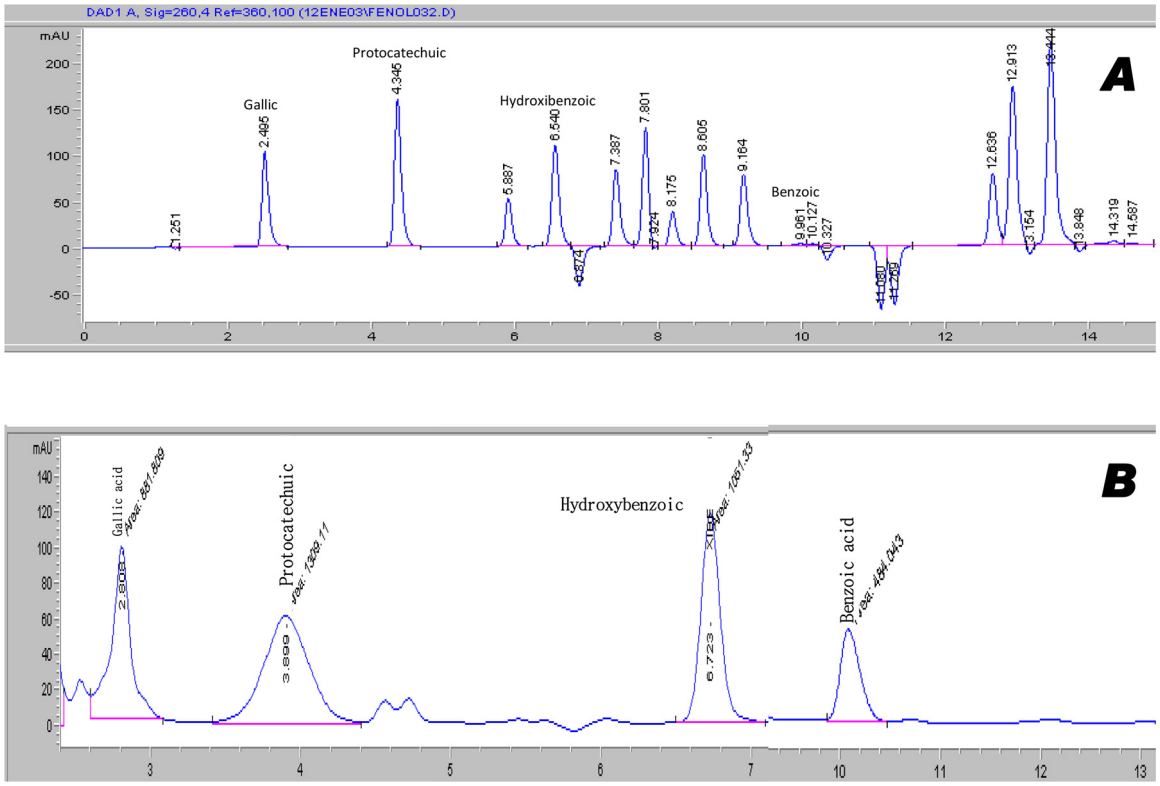

Fig. 2. Chromatogram for four out of 28 commercial standards of phenolic acids (A) and an amplification of the peaks and retention times for the gallic, protocatechuic, benzoic, and hydroxybenzoic acids detected in 'Roja Lisa' fruit stored at 10 °C for 112 d (B).

Table 3
Betalain and vitamin C concentrations (mg kg^{-1}) in 'Roja Lisa' and 'Amarilla Olorosa' cactus pears at harvest and stored under room temperature (RT) and cold room (CR) conditions. 'Amarilla Olorosa' and 'Roja Lisa' fruit were cold stored for 77 d and 112 d, respectively. Means ± standard deviation (n = 72). Values not detected are expressed as ND. Means in the same column with different letters indicate significant differences by Tukey's test at $p \leq 0.05$.

Main compound/variety/ compound	At harvest	Storage conditions at	
		RT (24 °C and 37 % RH for 35 d)	CR (10 °C and 95 % RH)
Betalains			
'Roja Lisa'			
Betacyanins	26 ± 3 c	55 ± 5 b	71 ± 5 a
Betaxanthins	10 ± 1 c	31 ± 5 b	46 ± 1 a
'Amarilla Olorosa'			
Betacyanins	2 ± 1 c	10 ± 3 b	12 ± 1 a
Betaxanthins	11 ± 1 c	43 ± 2 b	49 ± 2 a
Vitamin C			
'Roja Lisa'			
Ascorbic acid	369 ± 13 a	141 ± 7 b	154 ± 9 b
Dehydroascorbic acid	67 ± 2 a	ND	26 ± 2 b
'Amarilla Olorosa'			
Ascorbic acid	120 ± 7 b	123 ± 1 b	139 ± 3 a
Dehydroascorbic acid	ND	ND	ND

Consequently, there was more betacyanin than betaxanthin in 'Roja Lisa'. Regardless of pulp color, betacyanin and betaxanthin concentrations were greater in CR fruit than in RT and at harvest fruit in both cultivars (Table 3). Temperature is a crucial factor for betalain stability (Herbach et al., 2006; Cejudo-Bastante et al., 2016) and the concentration of these compounds increased with CR in both fruit cultivars. This suggest that a low VPD (10 °C and 95% RH) at CR, rather than a high VPD at RT (24 °C and 37% RH), increased betalains in both fruit cultivars. Similar behavior was observed when green, yellow, orange, red, and purple cactus pear fruits were stored at 10 °C and 60–70 % RH (Ramírez-Ramos et al., 2015) and in yellow pitaya, betaxanthin concentration increased along with time in storage (Cejudo-Bastante et al., 2016). The betaxanthin concentration was similar to that reported in orange (Albano et al., 2015) or yellow cactus pear fruit (Ramírez-Ramos et al., 2015; Debhi et al., 2013). Although betaxanthins are associated with yellow color, betaxanthin concentrations can be greater in some red cultivars than in yellow-orange ones, perhaps due to genotype-associated factors (Ramírez-Ramos et al., 2015; Khatabi et al., 2016). Betaxanthin concentrations in 'Roja Lisa' and 'Amarilla Olorosa' fruit are similar (Table 3).

The concentration of vitamin C (ascorbic and dehydroascorbic acids) was greater in red-pulped 'Roja Lisa' fruit than in yellow-pulped 'Amarilla Olorosa'. The greatest vitamin C concentration in 'Roja Lisa' fruit was observed at harvest and its concentration decreased by 58% or 62% in CR or RT fruit, respectively. However, the vitamin C concentrations reported here were greater than those reported previously (Albano et al., 2015). The concentration of dehydroascorbic acid had the same pattern, but was not detected in RT fruit, perhaps due to high VPD. In 'Amarilla Olorosa' fruit, the concentration of ascorbic acid was similar in at harvest and RT fruit, but increased in CR fruit, while dehydroascorbic acid was not detected (Table 3).

3.5. Antioxidant capacity (AC)

The AC is related to greater concentrations of vitamin C, phenolics, and betalains (Debhi et al., 2013; Albano et al., 2015). Although vitamin C decreased over time in both cultivars (Table 3), the AC increased due to the increase in phenolics and betalains. Cactus pear fruit color is directly related to the AC (Albano et al., 2015). Regardless of the storage condition, 'Roja Lisa' cactus pear had a greater AC than 'Amarilla Olorosa', except for TEAC at RT (Table 4). The AC was greater in fruit stored under CR conditions compared with at harvest values. In 'Roja Lisa' fruit, TEAC increased by 95% and 183% in RT fruit and CR fruit, respectively, compared to fruit at harvest. This increase was much larger in 'Amarilla Olorosa': TEAC, for instance, increased by 338% and 350% in RT fruit and CR fruit, respectively, compared to activity at harvest. Four methods were used to determine the AC to strengthen the results in this study, since TEAC is a more general and less specific method. The changes in TEAC in 'Roja Lisa' and 'Amarilla Olorosa' fruit were similar to DPPH, FRAP, and ORAC only in fruit at harvest and under CR conditions, and similar to that observed in red beet (Ravichandran et al., 2013). In general, this parameter was slightly enhanced at 10 °C, which may be related to a similar effect of temperature and RH detected for the phenolic compounds (Tables 1 and 2), which increased at 10 °C in both cultivars. Both phenol and betalain concentrations appear to have a synergistic effect with AC at low VPD, similar to that observed in nine cactus pear cultivars (Dehbi et al., 2013). Both cultivars had more AC than reported for purple and orange cactus pear fruit (Albano et al., 2015). 'Roja Lisa' fruit had more AC during storage (except for the TEAC method at RT) than 'Amarilla Olorosa' fruit (Table 4). The highest AC in 'Roja Lisa' fruit was coincident with high concentrations of TSP, TF, phenolic acids, vitamin C, and betacyanins (Tables 1,2, and 3). This association may explain, in part, its reduced FD (Lipińska et al., 2014) and extended storage life over 'Amarilla Olorosa' fruit (Fig. 1), despite the reduced CT in both fruit cultivars under both storage conditions (Table 1). Betaxanthins were reported as more active than betacyanins for AC in one study (Albano et al., 2015); however, our data suggest that 'Roja Lisa' fruit had the best AC, except for TEAC at RT storage (Table 4). Thus, the relationship between the increase of betalains and CT during RT and CR storage deserve further study.

4. Conclusions

'Roja Lisa' fruit had the lowest FML under both storage conditions. 'Roja Lisa' fruit could be kept under CR conditions for 5 weeks longer

Table 4
Antioxidant capacity (mmol kg^{-1} TE) in 'Roja Lisa' and 'Amarilla Olorosa' cactus pears at harvest and stored under room temperature (RT) and cold room (CR) conditions. Means ± standard deviation (n = 72). Means in the same column with different letters indicate significant differences by Tukey's test at $p \leq 0.05$.

Assessment condition/cultivar	TEAC	DPPH	FRAP	ORAC
At harvest				
'Roja Lisa'	8.82 ± 0.40 e	0.15 ± 0.30 d	0.14 ± 0.02 d	7.03 ± 0.40 b
'Amarilla Olorosa'	5.15 ± 0.18 f	0.06 ± 0.50 e	0.06 ± 0.01 e	5.79 ± 0.48 c
Stored at				
RT (24 °C and 37 % RH for 35 d)				
'Roja Lisa'	17.23 ± 0.25 d	0.21 ± 0.01 b	0.22 ± 0.01 b	7.90 ± 0.46 a
'Amarilla Olorosa'	22.60 ± 0.23 c	0.16 ± 0.01 cd	0.15 ± 0.004 d	6.20 ± 0.47 c
CR (10 °C and 95 % RH for 77 d for 'Amarilla Olorosa' and for 112 d for 'Roja Lisa')				
'Roja Lisa'	25.04 ± 0.85 a	0.30 ± 0.02 a	0.34 ± 0.03 a	8.59 ± 0.24 a
'Amarilla Olorosa'	23.19 ± 0.28 b	0.18 ± 0.01 c	0.19 ± 0.004 c	6.90 ± 0.14 b

than 'Amarilla Olorosa' fruit without reaching 8% FML or visible shrivelling. Total soluble phenolics and total flavonoids, and condensed tannins had, respectively, the highest and the lowest concentrations in CR stored fruit in both cultivars. The same pattern occurred in phenolic acids (gallic, protocatechuic, benzoic, and hydroxybenzoic), betalains (betacyanins and betaxanthins) vitamin C, and antioxidant capacity. The opposite occurred for dehydroascorbic acid, which was least in CR fruit of both cultivars and not-detectable in 'Amarilla Olorosa' fruit. Storing cactus pear fruit at CR or RT conditions for 5 weeks while fruit is transported to domestic or distant markets may enhance some nutraceutical properties that benefit consumers.

Acknowledgement

This research was partially supported by The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, research project No. Ref.: 8403134459. We thank Armando José María Carrillo Aguilera for his technical assistance. We thank Dr. Mary Lou Mendum (University of California) for improving the presentation of this document.

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