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ORIGINAL PAPER

Physicochemical quality, bioactive compounds and antioxidant activity of pitaya (*Hylocereus polyrhizus*) from Brazilian semi-arid during cold storage

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Abstract: The objective of this study was to assess the postharvest storage potential, quality, and antioxidant properties of pitaya (*Hylocereus polyrhizus*) subjected to cool storage conditions (10 ± 1°C and 90 ± 5% relative humidity). A completely randomized design was employed, with storage duration as the treatment factor (0, 7, 14, 22, and 32 days) and four replicates. Analyses were conducted on the fruits, including physical, physicochemical, and chemical assessments, as well as the determination of bioactive compounds and antioxidant activity using the ABTS method. Throughout the storage period under refrigeration, the pitaya maintained a favorable appearance and exhibited consistent sugar content (8.06% total and 7.55% reducing sugars). Furthermore, it demonstrated an increased soluble solids/titratable acidity (SS/TA) ratio at the conclusion of the storage period (78.11). The fruits remained commercially acceptable for up to 32 days, despite observed changes in fresh mass loss, external appearance, firmness, titratable acidity, and pigment concentrations. At the end of this period, they retained high rind firmness (40.03 N), pulp firmness (3.89 N), and substantial levels of betacyanins (58.00 mg per 100 g) and betaxanthins (91.97 mg per 100 g). These findings provide valuable insights for enhancing the shelf life and implementing effective measures to preserve the quality of pitaya fruit from the Brazilian semi-arid during cold storage and along the cold chain logistics.

Keywords: Functional foods, antioxidant activity, Cactaceae, conservation, exotic fruit, post-harvest shelf life.

Introduction

The pitaya (*Hylocereus polyrhizus*) is native to the tropical and subtropical regions of the Americas, spanning from southern Mexico to northern South America. It belongs to the Cactaceae family and is widely distributed across the entire American continent (Ortiz-Hernández and

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Carrillo-Salazar, 2012; Hua et al., 2018). Known by various names such as pitahaya, red pitaya, and dragon fruit, it is part of a group of fruits that hold great promise for cultivation.

Numerous cultivated fruits and species are commonly referred to as pitaya, and they are classified into four primary genera: *Stenocereus* Britton & Rose, *Cereus* Mill., *Selenicereus* (A. Berger) Riccob, and *Hylocereus* Britton & Rose (Britton and Rose, 1963; Nerd et al., 2002; Le Bellec et al., 2006). These genera exhibit variations in size, bract color, bract number, and soluble solids content (Mercado-Silva, 2018). The pitaya is an aesthetically appealing fruit known for its delightful taste, exotic appearance, and noteworthy nutritional and functional properties, rendering it a promising candidate for cultivation (Magalhães et al., 2019).

In Brazil, species of the *Hylocereus* genus hold significant economic importance and possess substantial exploration potential. This is especially true for the Brazilian semi-arid region due to their resilience, efficient water use, and year-round production capabilities. Among the species with considerable production and commercial potential, *H. polyrhizus* Britton & Rose stands out, characterized by its red rind and purple-red pulp teeming with numerous seeds (Le Bellec et al., 2006).

Researchers have classified pitaya as a non-climacteric fruit, meaning it achieves its highest quality when fully ripe (Li et al., 2017). However, pitaya fruit has a short shelf life, and exposure to room temperatures during marketing and storage can lead to undesirable senescent changes, such as rapid shriveling and a loss of bract greenness (Chaemsanit et al., 2018). Given the tropical nature of pitaya, storage conditions need to be carefully managed, as most pitaya species are not tolerant to temperatures below 10°C, where the growth of plant is impaired (El-Ramady et al., 2015).

Before introducing a fruit, such as pitaya, to the market, it is essential to characterize its postharvest changes (García-Cruz et al., 2016). When targeting foreign markets, as is the case with pitaya, it becomes crucial to employ postharvest technologies to enhance the fruit's shelf life. Cooling is one of the most effective and widely used methods for reducing the metabolic activity of fruits, thereby preserving their attractiveness and suitability for consumption over a longer period.In addition to that, storage tolerance is species-, genotype-, and origin-dependent.

For pitaya of the *H. undatus* variety grown in California, the optimal storage temperature is 5°C (Freitas and Mitcham, 2013). In São Paulo State, in the Southeast region of Brazil, shelf life can be extended to 25 days at temperatures ranging from 8 to 13°C (Brunini and Cardoso, 2011). In the Southern region (Marinalva, Paraná State), however, the chemical composition of the pitaya fruits decreased after 12 days of refrigeration at 5 ± 1°C, with 46 and 65% relative humidity (minimum and maximum, respectively). Similarly, pitaya fruits from Vietnam, stored for over three weeks at 5°C, exhibited almost no flavor loss and maintained acceptable outer quality (Hoa et al., 2006). In Chapada do Apodi (Apodi, Rio Grande do Norte State), pitaya fruits demonstrated good physical, chemical and nutritional characteristics to be consumed in nature (Nunes et al., 2014). However, to date, there is a lack of data regarding pitaya from the Brazilian Northeast stored under refrigeration. This is particularly important for regions like Fortaleza, (Ceará State), Mossoró, (Rio Grande do Norte State), and São Paulo, (São Paulo State), where a well-established cold chain for melon and papaya transport utilizes temperatures ranging from 10 to 13°C. In light of the above, the objective of this study was to assess the preservation potential, quality, and antioxidant capacity of red-purple pitaya (*H. polyrhizus*) from the Brazilian Northeast semi-arid region.

Materials and Methods

Plant material and experimental design

Red-purple pitaya fruits of the *Hylocereus polyrhizus* species, characterized by their red peel (epicarp) and red-purple pulp, were sourced from a commercial orchard that had been established for three years. This orchard is situated within the irrigated perimeter of Jaguaribe-Apodi, in the municipality of Limoeiro do Norte (05° 08' 45" S, 38° 05' 52" W), Ceará State, Brazil, located in the Brazilian semiarid region. The climate in this area, classified according to the Köppen system, is "BSwh", signifying a dry and very hot climate with two distinct seasons: a dry season typically spanning from June to January and a rainy season from February to May (Alvares et al., 2013). During the reproductive months leading up to the harvest (May to August 2014), the climatic conditions in the region were as follows: 68.77% relative humidity, mean temperature of 26.64°C, and precipitation of 59.80 mm (UEPE Meteorological Station – Teaching, Research, and Technological Extension Unit/Chapada do Apodi, Instituto Federal de Educação, Ciência e Tecnologia do Ceará – IFCE, Limoeiro do Norte Campus).

The fruit harvesting process was conducted manually in the morning, selecting fruits at a maturation stage suitable for commercial purposes, characterized by uniform red coloring of the peel throughout the fruit, adhering to company guidelines. These harvested fruits were then transported to the Laboratory of Post-Harvest Physiology and Technology of Fruits at the Federal University of the Semi-Arid Region (UFERSA), Mossoró (5°11'16" S, 37° 20' 38" W), Rio Grande do Norte, Brazil. In the laboratory, the fruits underwent a selection process, where any fruits displaying damage from cuts, abrasions, insect or animal attacks were discarded. Subsequently, the selected fruits were placed in polystyrene trays (23.5 × 18.0 × 1.5 cm) and stored in a cooler maintained at 10 ± 1°C and 90 ± 5% relative humidity, marking the beginning of the experiment. These conditions are already used in melon storage in the region.

The experiment was designed following a completely random design, with the evaluation times serving as the treatments (harvest – 0, 7, 14, 22, and 32 days), with four replicates (experimental units). Two fruits were used per replicate, totaling eight fruits per each treatment. For all the fruits, physical analyses were performed, and they were subsequently destroyed for further analysis.

For the analysis of betacyanins and betaxanthins, a factorial design of 3 × 5 was employed, with the first factor being three types of extractors (water, 70% ethanol, and 80% ethanol), and the second factor representing the five storage times. The inclusion of three different extraction solvents aimed to evaluate their extraction efficiency across different stages of fruit degradation during storage. Crucially, all solvent extractions at each time point were performed using subsamples derived from the same biological replicates used for the overall storage evaluations, ensuring that observed variations were due to extraction capacity rather than biological diversity between samples.

To assess the physical quality of the fruits, the pulp, including the seeds, was separated from the peel (epicarp) after transversally cutting the fruit manually, aided by stainless steel knives. The pulp fraction (mesocarp + seeds) was homogenized using an T 25 Ultra-Turrax® type tissue homogenizer (IKA Brazil, Campinas, SP, Brazil), forming a single sample, which was then packed in plastic containers and stored in a freezer at -23°C for subsequent analysis. Additionally, photographs were taken at each storage period to visually demonstrate the overall appearance (Figure 1).

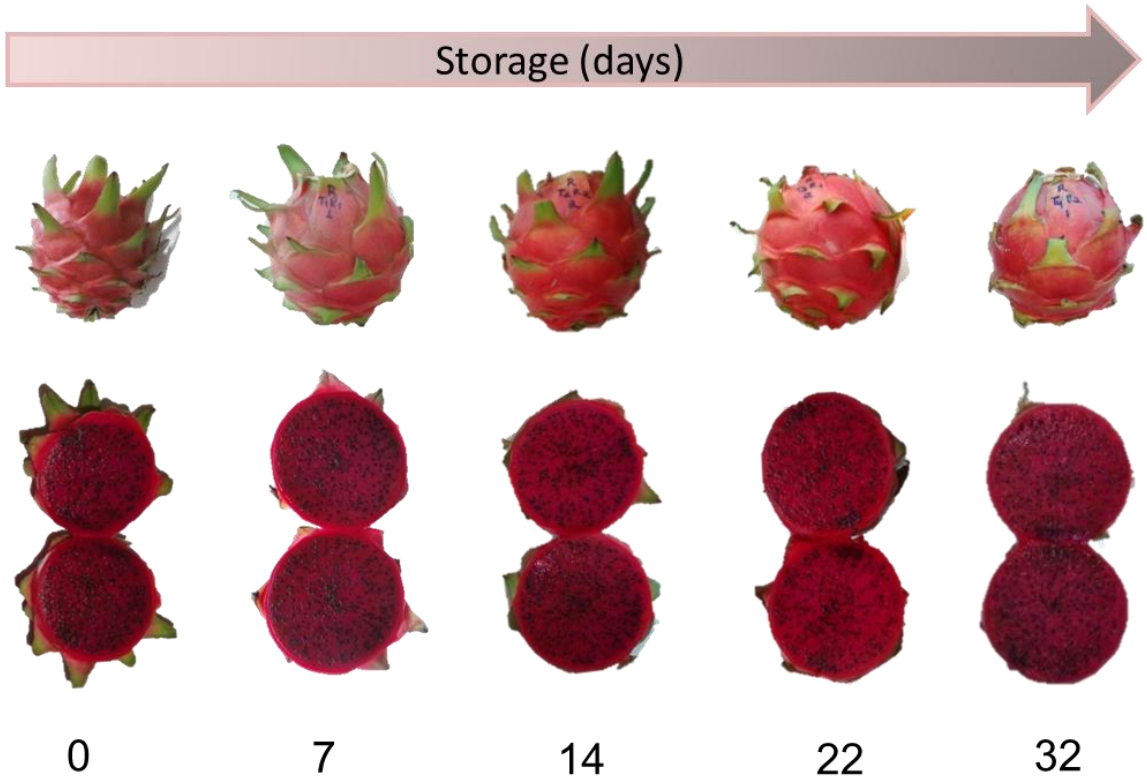

Figure 1. Pitaya fruits stored for 32 days at 10 ± 1°C and 90 ± 5% relative humidity.

Physical characteristics

The longitudinal (mm) and transverse (mm) diameters were meticulously determined using a precise digital caliper (Lotus Plus, Shan, Shanxi, China). To quantify the fresh weight loss (g) of the fruits, a highly accurate semi-analytical balance model Mark L 1002 (Bel Equipamentos Analíticos Ltda., Piracicaba, SP, Brazil) was employed. These measurements were taken at the outset of the experiment (day 0) and at distinct intervals during the storage duration. The yield of pulp (%) was calculated by computing the disparity between the total mass of the fruit and the mass of the peel.

A panel of three trained evaluators subjectively assessed the external appearance of the fruits. Prior to the evaluations, the panel underwent a calibration training session where they were presented with reference images and physical samples representing the different defect levels (0 to 4) to standardize the visual perception of characteristics. These scores were determined based on the severity of defects observed on the fruit's surface, encompassing factors such as bracts wilting (manifesting as scales curving inward), alterations in color, peel depressions, the presence of spots, and the impact of microorganism-related damage, collectively defined as defects. Specific criteria were established: scores of 4.0 to 3.1 indicated fruits in excellent market condition (exhibiting defects on less than 5% of the fruit surface and bracts); scores of 3.0 to 2.1 signified fruits in good market condition (with defects covering 5 to 25% of the fruit surface); scores of 2.0 to 1.1 represented fruits in moderate market condition (with 26 to 50% of the fruit surface displaying defects); scores of 1 to 0 denoted fruits lacking market viability (with over 50% of the fruit surface affected by defects). A score below 1.5 served as the threshold for categorizing fruits as non-marketable. It is important to note that this scoring system drew inspiration from the work of Woolf et al. (2006), and was further adapted from Brunini and Cardoso (2011).

The color characteristics of both the peel and pulp were quantified using the CIELab color space, which measures three key parameters: L (luminosity – brightness, clarity, or reflectance), C* (chroma – saturation or color intensity), and °h (hue angle – tonality). This evaluation was conducted with the assistance of a CR-410 digital benchtop colorimeter Minolta® (Chiyoda-ku, Tokyo, Japan). To obtain these measurements, the following procedures were followed: peel color (readings were randomly taken at two equidistant points located in the equatorial region of the fruit's peel; pulp color (after making a transversal cut of the fruit, measurements for the pulp color were conducted at the center of both pulp sections, with the final result being an average of the two measurements).

The assessment of fruit and pulp firmness was performed using the TA.XTExpressC Texture Analyzer® (Stable Micro Systems Ltd., Godalming, Surrey, England). A 10 kg load cell was utilized in the procedure and a cylindrical stainless steel probe with 6 mm diameter model P/6 (Stable Micro Systems Ltd., Godalming, Surrey, England) was chosen. The penetration depth inside the fruit was 30 mm. The pre-test, test, and post-test velocities used in this procedure were 2, 2, and 10 mm s^{-1}, respectively. In the equatorial region of the fruit and for the pulp, two equidistant measurements were taken. For the pulp, these measurements were made after a transversal cut of the fruit, at the center of both pulp sections, and the average of the two measurements was considered. In total, four measurements were taken in each fruit. The results of these firmness measurements were expressed in Newtons (N).

Physicochemical and chemical characteristics

The hydrogenation potential (pH) was determined using a direct reading potentiometer model mPA-210 (Tecnal®, Piracicaba, SP, Brazil) duly standardized with buffer solutions pH 7.0 and pH 4.0 (Association of Official Analytical Chemists – AOAC, 2016) in 5 g aliquots of the pulp diluted in 50 mL of distilled water, that after the stabilization of the results, the data were expressed in real pH values (AOAC, 2016). The titratable acidity (TA) was determined by electrometric procedure using 5 g of pulp transferred to a 125 mL Erlenmeyer flask with the aid of 50 mL of water, then titrating with 0.1 N NaOH solution (AOAC, 2016), using an automatic titrator model Class A (Titrette® BRAND, Werthein, Baden-Württemberg, Germany), with results expressed as mg of malic acid per 100 g of pulp. The soluble solids (SS) were determined with the homogenized juice of the pulp after being filtered in organza type fabric in a digital refractometer model PR-100 (Atago Co., Ltd., Fukaya-shi, Saitama, Japan) (AOAC, 2016), the results were expressed as a percentage (%). Based on the SS and TA data, the SS/TA ratio was calculated.

The chemical analysis of the red-purple pitaya pulp encompassed the determination of both total sugars and reducing sugars, employing specific methods and analytical instruments.

Total sugar content in fresh mass was ascertained using the anthrone method, involving the utilization of anthrone (Vetec Química Fina Ltda., Duque de Caxias, RJ, Brazil). For each fruit, three replicates were used. To initiate this analysis, a 0.5 g sample of the pulp was used to prepare an extract. Subsequently, a 100 µL aliquot of this extract was withdrawn for spectrophotometer readings at a wavelength of 620 nm. These readings were carried out using the UV-1600 model spectrophotometer (Pro-Analysis®, Belém, PA, Brazil). The results obtained were expressed as a percentage (%). The quantification of reducing sugars in fresh mass was executed in accordance with the DNS (3,5-dinitro salicylic acid) method, following the protocols outlined in the AOAC (2016) guidelines. For each fruit, three replicates were used. The process

involved the extraction of the reducing sugars from 1 g of the pulp, from which 0.45 mL was extracted. To this volume, 1.05 mL of distilled water and 1 mL of 1% dinitro salicylic acid – DNS (Vetec Química Fina Ltda., Duque de Caxias, RJ, Brazil) were added. The reaction was then conducted in a water bath at 100°C for 5 min, followed by cooling in an ice bath. Subsequently, spectrophotometer readings were performed at a wavelength of 540 nm. The results were expressed as a percentage.

Bioactive compounds and total antioxidant activity

The quantification of vitamin C involved titration with a 0.02% dichlorophenol-indophenol solution (Strohecker and Henning, 1967). A total of 2.5 g of the pitaya pulp samples were utilized. These samples were then diluted in a 100 mL volumetric flask with a 0.5% oxalic acid solution. Subsequently, 5 mL of this solution were further diluted with distilled water to reach a final volume of 50 mL, and the titration was executed. The results were expressed as mg of ascorbic acid per 100 g of fresh matter (FM).

For the determination of total anthocyanins and yellow flavonoids were determined according to Francis (1982). In this analysis, 1 g samples of the pitaya pulp were mixed in a 50 mL extractive solution composed of 95% ethanol and 1.5 N HCl in an 85:15 v/v ratio. This mixture was homogenized and stored at 4°C for 12 h. Spectrophotometer readings UV-1600 model (Pro-Analysis®, Belém, PA, Brazil) with a wavelength of 374 nm for flavonoids and 535 nm for anthocyanins were used. The results were expressed mg per 100 g FM.

Betacyanin and betaxanthin contents were determined using extracts prepared by homogenizing 1 g of pulp in 10 mL of different solvents (water, ethanol:water in proportions of 70:100 v/v and 80:100 v/v) for 30 min. The resulting filtrate was diluted, when necessary, with 0.05 M citrate-phosphate buffer (pH 6.5) to ensure that the spectrophotometric absorbance (A) remained within the linear range of $0.8 < A < 1.0$. Readings were performed at 538 nm for betacyanins and 480 nm for betaxanthins (Stintzing et al., 2003; Cejudo-Bastante et al., 2016). Any analysis regarding anthocyanins, betaxanthins and betalains content was interpreted with caution, as betacyanins exhibit significant spectral overlap near 535-540 nm.

Extracts for total extractable polyphenols and total antioxidant capacity

The procedure developed by Rufino et al. (2010) was used and is described as follows: 17.5 g of the samples were weighed into centrifuge tubes and sequentially extracted with 10 mL of methanol/water (50:50 v/v) at room temperature for 1 h. The tubes were centrifuged at 10,000 rpm for 20 min and the supernatant recovered. Then, 10 mL of acetone/water (70:30 v/v) was added to the residue at room temperature, extracted for 60 min and centrifuged. Extracts of methanol and acetone were mixed in a volumetric flask and added to 25 mL of distilled water. The extract was used to determine the content of total extractable polyphenols and antioxidant capacity.

Polyphenols were determined by colorimetric assay using Folin-Ciocalteu reagent, according to the methodology described by Obanda et al. (1997). This assay was performed in triplicate for each replicate. The samples were extracted with 50% methanol and 70% acetone. The determination was performed using aliquots of 150 µl of the extracts in test tubes and to which were added 850 µL of distilled water, 1 mL of Folin-Ciocalteu reagent, 2 mL of 20% sodium carbonate solution and 2 mL of distilled water. The samples were then shaken in a tube shaker QL-901 model (Vortex®, São Paulo, SP, Brazil) and allowed to stand for 30 min in the dark. The readings were spectrophotometer at 700 nm, using the standard curve of gallic acid 98% (dosed at 0, 10, 20, 30, 40, and 50 µg).

The results were expressed as gallic acid equivalents (GAE) mg per 100 g FM.

Total antioxidant activity - ABTS•+ assay

The total antioxidant activity (TAA) was determined using 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid radical cation (ABTS•+, Sigma), the method described by Re et al. (1999). This assay was performed in triplicate for each replicate. Before the colorimetric assay, the samples were subjected to an extraction procedure in 50% methanol and 70% acetone (Rufino et al., 2010). The ABTS•+ radical was generated by reacting the 7 mM ABTS solution with 140 mM potassium persulfate leaving in the dark at room temperature for 16 h. Once the ABTS•+ radical was formed, it was diluted with ethanol until an absorbance value of 700 nm ± 0.05 at 734 nm was obtained. The spectrophotometric reading was done after 6 min from the mixture of 30 μL of extract with three mL of the ABTS•+ radical, using the synthetic antioxidant Trolox in the concentration of 100–2000 μM in ethanol to prepare the calibration curve. The results were expressed in μmol Trolox per g FM.

Statistical analysis

Data were submitted to analysis of variance by the F-test ($p \leq 0.05$). Data on evaluation times were subjected to polynomial regression (linear and quadratic), selecting the models according to its significance, its determination coefficients, and biological phenomenon. The means were separation using Tukey's test ($p \leq 0.05$). The statistical analysis was performed with the SISVAR 5.6 statistical program (Ferreira, 2011).

Results and Discussion

Physical characteristics

The pitayas utilized in this study exhibited an average mass of 485.73 g, with a longitudinal length of 95.53 mm and a transversal length of 94.16 mm. These fruits were characterized by a rounded shape, as reported by Lima et al. (2014). It's worth noting that these pitayas possessed a higher fresh weight in comparison to the *Hylocereus. undatus* variety (Hoa et al., 2006; Brunini and Cardoso, 2011), which is the most widely cultivated, commercialized, and widely accepted species among consumers on a global scale. Furthermore, the fruit weight fell within the acceptable range for exportation standards, typically spanning from 350 to 700 g fruit^{-1} (Woolf et al., 2006; Mercado-Silva, 2018). These attributes are of paramount significance as they impact consumer preferences and overall fruit yield.

During the course of refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity), a significant effect of storage time was observed on external appearance ($p < 0.01$), fresh mass loss ($p < 0.01$), pulp yield ($p < 0.01$), peel thickness ($p < 0.01$), fruit firmness ($p < 0.05$), and pulp firmness ($p < 0.01$) of the pitaya fruit. These findings underscore the influence of storage duration on the overall quality and attributes of the pitaya fruit under refrigerated conditions.

The visual appearance of the fruits maintained their highest scores until the 21st day of storage, with an overall rating of approximately 3.1. At this point, the fruits exhibited only minimal defects, with wilting affecting about 5% of them, along with some slightly curved bracts, loss of the original color, and minor issues such as scale and surface rotting (Figure 2A). However, beyond this timeframe, the fruits received a reduced score of 2.4, categorizing them as fruits with good appearance and suitable characteristics for the market. It's noteworthy that in many final markets, the appearance of pitaya is often compromised, particularly due to wilting issues (Mizrahi, 2014). The primary defects that influenced the quality of pitaya in this study were wilting, bracts wilting with moderate curving, and an unusual color formation affecting approximately 25% of the fruit's surface. As noted by Brunini and Cardoso (2011), the external appearance of pitaya is influenced by temperature and storage duration. Quality

deterioration during storage can be attributed, in particular, to temperature fluctuations, which accelerate the respiration process, thereby initiating senescence. This characteristic emphasizes the importance of external appearance as one of the most critical fruit quality parameters. It plays a pivotal role in determining the commercial value of the product, especially for fruits intended for the fresh market.

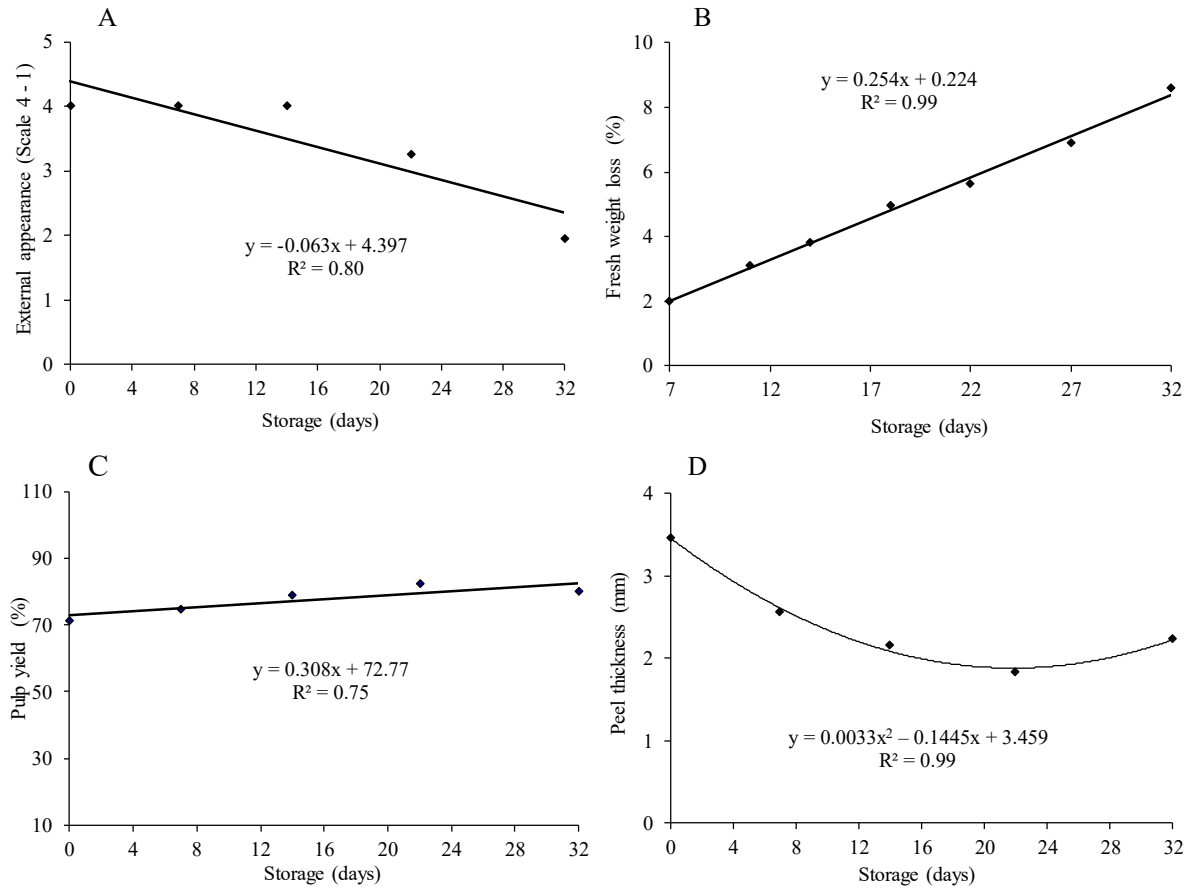

Figure 2. External appearance (A), fresh weight loss (B), pulp yield (C), and peel thickness (D) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

Regarding fresh mass loss, the pitayas exhibited their highest values, reaching 8.6%, on the 32nd day of storage (Figure 2B). Although this level is below the critical threshold of 10% considered detrimental to fruit appearance (Chitarra and Chitarra, 2005), it did result in some wrinkling of the fruit's skin. In comparison, Brunini and Cardoso (2011), in their study on the shelf-life of pitaya (*H. undatus*) at 13°C, observed a slightly higher mass loss of 7.82% within the first 25 days of storage, surpassing the results obtained in the current research during the same timeframe. These findings are consistent with the assertion made by Brunini and Cardoso (2011) that fresh mass loss during fruit storage is a critical factor affecting both commercialization and preservation. It can lead to a devaluation of the product in the market due to issues such as skin wrinkling and wilting, even though the pulp of the fruit generally remains in good condition. On the other hand, Hoa et al. (2006) reported that pitaya of the *H. undatus* species, when stored for 2 to 4 weeks at 5°C in polypropylene bags, exhibited mass loss that was not significant over the course of storage. A similar behavior was also observed by Santos et al. (2016) in their study of pitaya fruit

evaluated up to 12 days of refrigeration at 5 ± 1°C, with 46 and 65% relative humidity (minimum and maximum, respectively).

A notable linear increase in pulp yield was observed during the storage period (Figure 2C). At the time of harvest, the fruits had a pulp yield of 72.77%. By the end of the storage period, this yield had increased to 82.77%, indicating a significant rise of 11.93%. This increase is likely associated with two key factors: the fresh mass loss (Figure 2C) and a decrease in peel thickness (Figure 2D) by 36.98%. This decrease in peel thickness, as explained by Abreu et al. (2012), can be attributed to an osmotic pressure gradient, which results in a higher sugar concentration in the pulp compared to the rind. This gradient, in turn, facilitates the movement of water from the peel to the flesh during the maturation process. This phenomenon may have contributed to the lower fresh mass loss during storage, ultimately preserving the appearance of the pitayas.

Compared to other dragon fruit varieties, pitaya fruits demonstrate a notably higher pulp content, a characteristic that holds significant value for both fresh consumption and processing into various products (Cordeiro et al., 2015). Various studies have reported pulp yields in different regions, such as 71% in fruits from Nicaragua (Vaillant et al., 2005), 63.37% in Malaysian pitayas (Lim et al., 2010), up to 81.03% in pitayas from the Pará State in Brazil (Sato et al., 2014), and 75.25% in pitayas from the Minas Gerais State in Brazil (Cordeiro et al., 2015). An mean yield of 75.10% of the total weight was observed for 21 pitaya accessions from the *H. undatus* and *Selenicereus setaceus* species in the Cerrado in South region of the Brazil (Lima et al., 2014). It's worth noting that the peel of the pitaya constitutes approximately 21.10 to 31.90% of the entire fruit. Unfortunately, this peel is often discarded, resulting in a considerable loss of pectin, betacyanin pigments, and valuable dietary fiber. The pitaya peel can potentially serve as a rich source of these nutrients and natural dyes (Fathordoobady et al., 2021).

The fruit firmness exhibited a noticeable decline throughout the storage period. At the time of harvest, the fruits displayed an mean firmness of 51.45 N, which decreased to 40.03 N by the end of the storage period (Figure 3A), marking a reduction of 22.20%. This decrease in firmness renders the fruits more susceptible to mechanical impacts and damage that can occur during their shelf life. A similar reduction in firmness was observed by Brunini and Cardoso (2011) in fruits stored at temperatures of 8 and 18°C, and this decrease was found to be influenced by both temperature and storage duration. Values of 44.31 N were reported for fully ripe red pulp pitaya (Cordeiro et al., 2015).

The loss of firmness is typically associated with the degradation of cell wall compounds during fruit maturation (Pareek, 2016). Even though the fruits were harvested at a stage suitable for consumption, the observed variation in firmness indicates that modifications in polysaccharides within the middle lamella and primary cell wall can still occur during the senescence phase (García-Cruz et al., 2016). According to authors, who assessed the biological and physical characteristics of pitaya from different species, the fruit generally possesses a soft consistency, making it vulnerable to mechanical damage. The loss of turgescence may play a significant role in diminishing the mechanical resistance of fruit tissue. Thus, it is of paramount importance for varieties to exhibit higher firmness, as this would enhance their resistance to damage and potentially prolong their shelf life.

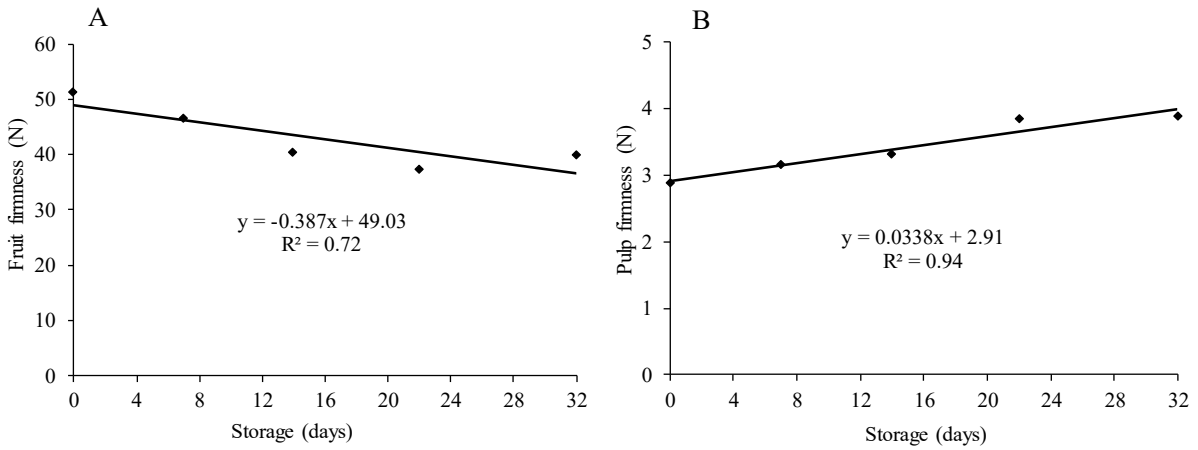

Figure 3. Fruit firmness (A) and pulp firmness (B) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

During refrigerated storage, there was a notable increase in pulp firmness (Figure 3B). The values went from 2.88 N at harvest to 3.16 N at 7 days, 3.31 N at 14 days, 3.84 N at 22 days, and finally, 3.89 N at 32 days, marking a total increase of 26.01% by the end of the storage period. This increase in pulp firmness aligns with findings reported by Brunini and Cardoso (2011) for pitaya stored at 13°C for 25 days at 85-90% relative humidity. Such behavior may be associated with the phenomenon of epicarp drying, which is brought about by water loss (García-Cruz et al., 2016). This drying leads to withering and tissue flaccidity, making it difficult for a penetrometer to penetrate the pulp (Brunini and Cardoso, 2011). Results have shown variations in pulp firmness, ranging from 6.16 N (Menezes et al., 2015), 6.30 N (Yah et al., 2008), 13.70 N (Hoa et al., 2006), and from 1.14 to 1.87 N (García-Cruz et al., 2016), for different species of pitaya fruits when fully ripe.

Significant effects were observed for all the peel color variables, including luminosity, chromaticity, and hue angle ($p < 0.05$). The mean values for chromaticity, hue angle, and luminosity were 40.31, 21.12°, and 47.08, respectively. The fruit's color saturation was highest on the 19th day of storage (43.20) and lowest at harvest (35.95) (Figure 4A). The hue angle displayed a decreasing trend during storage, with the lowest value of 18.70° on the 24th day. This shift began with a predominantly bright red color (25.49°) and transitioned to an intense red (19.75°). The peel color underwent changes during storage, gradually shifting from orange-red to red and ultimately to purple-red (Cruz et al., 2015). A similar trend was observed for luminosity, with a gradual darkening (reduction in peel brightness). The lowest luminosity value (44.81) was recorded on the 25th day, corresponding to a reddish pulp (Figure 4A), with a darker color and reduced brightness. These changes in color parameters align with findings by Obenland et al. (2016), who noted that the luminosity of four pitaya varieties decreased when stored at 10°C, indicating a subtle darkening of color, an increase in chroma, and a decrease in hue angle.

Regarding pulp color, there was a significant effect for chroma, which increased during refrigerated storage (Figure 4B). On average, chromaticity, hue angle, and luminosity values were 28.60, 1.21°, and 27.60, respectively. The chroma values indicated a higher color saturation in the pitaya pulp at the end of storage. In the case of *H. polyrhizus*, the accumulation of pulp pigments coincided with the development of peel color (Figure 4A), resulting in a red hue that tended toward purple (purple-red). These colors fall within the spectrum of shades ranging from red to blue, consistent with the existing literature on this species (Nerd et al., 2002; Le Bellec et al., 2006; Obenland et al., 2016).

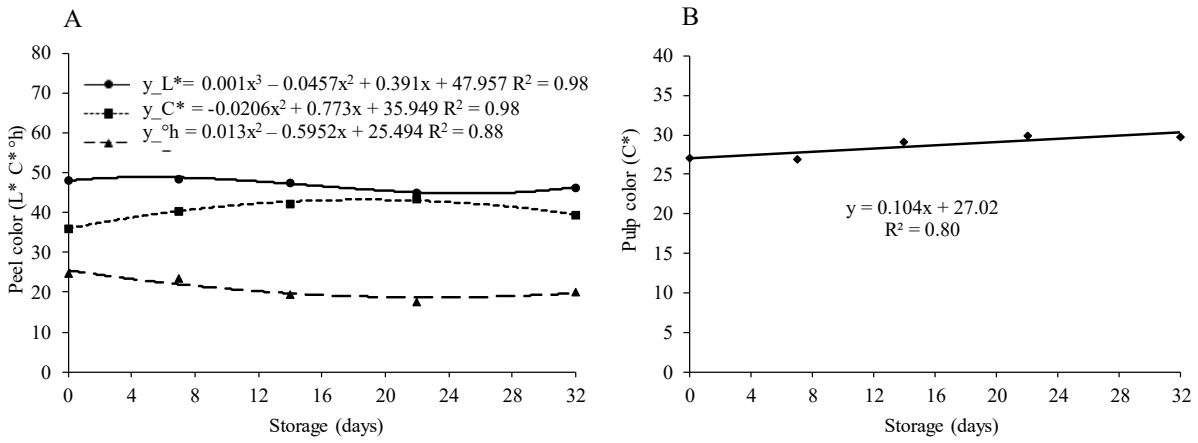

Figure 4. Peel luminosity (L*), chromaticity (C*), and hue angle (°h) (A) and pulp color (chromaticity) (B) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

The values of pulp chromaticity and hue angle were in line with those reported by Chik et al. (2011), who found values of 27.80 and 3.10° for *H. polyrhizus* from Malaysia, indicating an intense red color. In contrast, Cordeiro et al. (2015) observed pitayas produced in Janaúba-MG, Brazil, with values of 38.89 and 14.25° for chromaticity and hue angle, respectively, which represented an intermediate color between red and yellow. Therefore, the fruits in this study exhibited higher color saturation, making them more visually appealing. The purple-red pulp observed in these pitayas is considered an attractive feature for their use as ingredients in various food products (Mizrahi, 2014; Sato et al., 2014), with betacyanins being the primary pigments responsible for this coloration (Stintzing et al., 2004; García-Cruz et al., 2013).

Physicochemical and chemical characteristics

In the physicochemical analyses, a significant effect ($p < 0.01$) of refrigerated storage was observed for soluble solids(SS), titratable acidity (TA), SS/TA ratio, and pH. However, there were no significant differences in total and reducing sugars during storage ($p > 0.05$). The highest soluble solid content was recorded at harvest (12.84%), while the lowest was observed at the end of the storage period (10.22%) (Figure 5A), representing a total decrease of 20.44%.

These findings are consistent with previous studies. García-Cruz et al. (2016) reported soluble solid concentrations ranging from 9 to 11% in different pitaya species. Obenland et al. (2016) observed soluble solid contents between 10.89 and 13.29% for different cultivars. Cordeiro et al. (2015) found a content of 13.14% for *H. polyrhizus*. Additionally, Yah et al. (2008) and Enciso et al. (2011) reported soluble solid contents ranging from 11.60 to 13.60% for *H. undatus*. Lower storage temperatures contribute to better preservation of soluble solids by reducing metabolic processes. This was evident from the decrease in soluble solid content, with a reduction from 25.91 to 24.17% in *H. undatus* stored at 18 and 13°C for 15 and 25 days, respectively (Brunini and Cardoso, 2011).

The titratable acidity exhibited a consistent decrease during the storage period (Figure 5B). The acidity levels were measured at 0.39, 0.34, 0.28, 0.22, and 0.14 mg of malic acid per 100 g of pulp at harvest, 7, 14, 22, and 32 storage days, respectively, marking a significant decline of 65.14%. This trend was mirrored by the pH values, as the decrease in acidity corresponded to an increase in pH over the storage period. The pH levels were measured at 4.64 at harvest and 5.76 on the

32nd storage day (Figure 5C), indicating a notable rise of 19.49%.

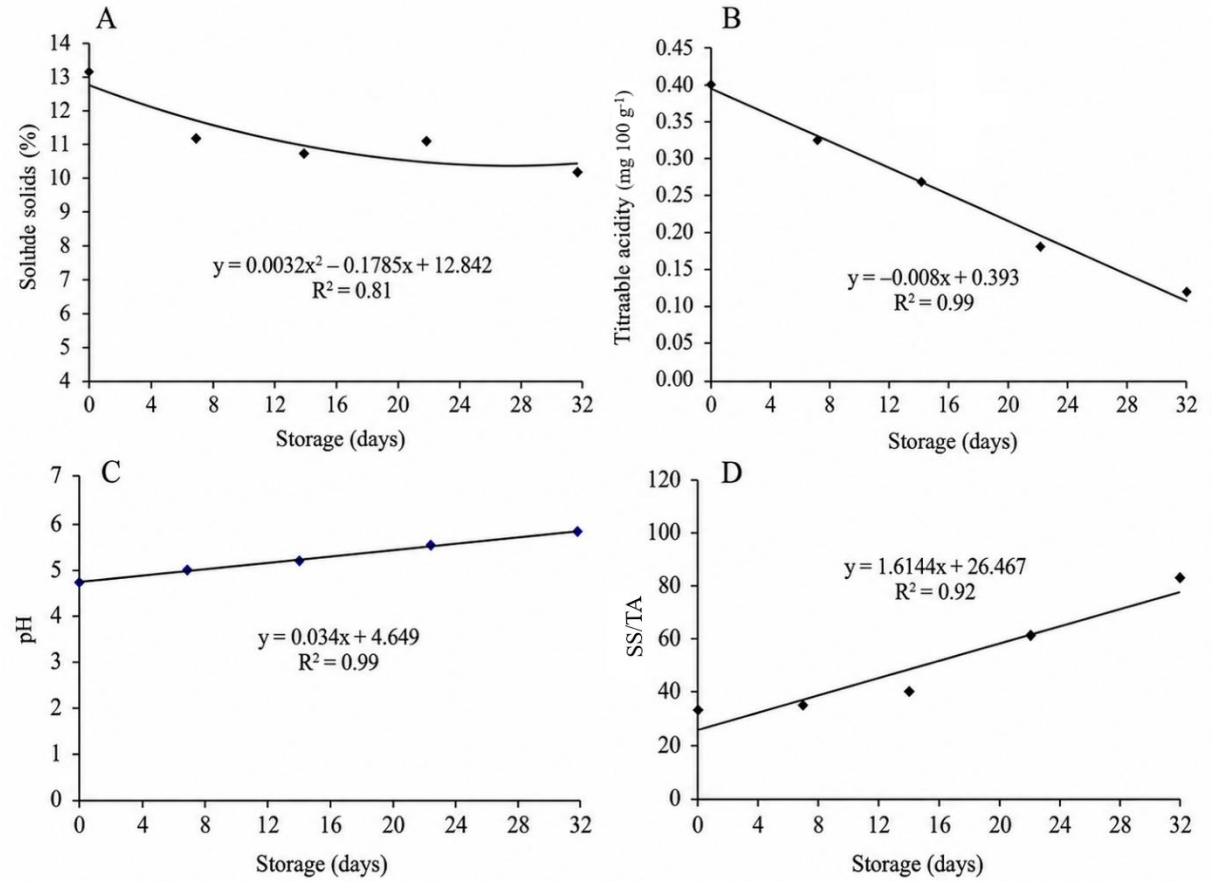

Figure 5. Soluble solids – SS (A), titratable acidity – TA (B), pH (C), and SS/TA ratio (D) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

A similar drop in acidity, amounting to a significant 79.50%, was documented by Freitas and Mitcham (2013) in pitayas stored at 10°C. They recorded an acidity level of 0.36 mg per 100 g at harvest, which decreased to 0.074 after 20 days of storage. Previous studies have consistently reported that fresh pitayas typically have low acidity content ranging from 0.07 to 0.24 mg per 100 g (Brunini and Cardoso, 2011; Abreu et al., 2012; Cordeiro et al., 2015). This acidity reduction is known to accelerate with time, depending on storage conditions (Brunini and Cardoso, 2011), and is a result of the fruit utilizing organic acids as an energy source during respiration, which can impact the fruit's color, flavor, and overall quality (Lima et al., 2013).

According to García-Cruz et al. (2016), even though the pitaya's respiration rate appears to be low, it is sufficient to cause a reduction in both soluble solids and acidity content, as demonstrated in Figures 4A and 4B, respectively. These observations align with the findings in literature, such as pH of 4.88 (Abreu et al., 2012), pH ranging from 4.80 to 5.70 (Lima et al., 2013), pH of 5.32 (Cordeiro et al., 2015), and pH between 4.61 and 5.13 (Santos et al., 2016). These studies collectively illustrate the effect of respiration on pH and the resulting changes in soluble solids and acidity levels during pitaya storage.

The decrease of acidity resulted in an increase of 195.19% on the SS/TA ratio during the whole storage, going from 26.46 at harvest day to 78.11 at the storage end

(Figure 5D). These values of SS/TA were highest to the obtained in pitaya *H. polyrhizus* (45.31) produced in the Minas Gerais State Semiarid (in Brazil) (Cordeiro et al., 2015) and in São Paulo State (51.90) (*H. undatus*) (Brunini and Cardoso, 2011), as well as in different pitaya genotypes (21.35 to 40.90) studied by Obenland et al. (2016). When compared to other commercial fruits, the SS/TA ratio of pitaya is higher than what is found for mangos 'Van Dyke' (71.26), 'Tommy Atkins' (49.55) and 'Keitt' (46.51), West Indian cherry (4.22–7.45), guavas 'Paluma' (18.87), 'Rica' (22.47) and 'Pedro Sato' (25.52) (Batista et al., 2015).

The total sugars and reducing sugars did not exhibit significant variations over the storage period, with overall mean values of 8.06 and 7.53%, respectively (Table 1). Notably, the proportion of reducing sugars present in the pulp was relatively high, accounting for 93.67% of the total sugars. This high proportion of reducing sugars is a desirable characteristic in fruits as it contributes to a sweeter taste. Consistent with the findings of this study, Santos et al. (2016) observed a decrease in both total and reducing sugar content during a 12-day storage period at 5 ± 1°C with relative humidity ranging from 65 to 46%. García-Cruz et al. (2016) also reported a reduction in sugar content after storing fruits for 10 days at 26°C and 90% relative humidity. The stable sugar content throughout storage is advantageous for maintaining fruit quality, especially concerning flavor. For comparison, Cordeiro et al. (2015) reported mean values of 8.79% for total sugars and 5.56% for reducing sugars in *H. polyrhizus*, while Santos et al. (2016) found values of 2.67% for total sugars and 8.83% for reducing sugars in *H. undatus*. The results of the variables here evaluated (Figures 2, 3, and 4, and Table 1) indicate that, despite being different species, the values are similar to *H. undatus*, the most cultivated and marketed pitaya worldwide, favoring the acceptability of *H. polyrhizus* produced in the Brazilian semi-arid, by consumers.

Table 1. Total and reducing sugar average of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

Storage time (days)	Variable	
	Total sugars (%)	Reducing sugars (%)
Harvest	7.95a	7.56a
7	7.79a	7.31a
14	7.96a	7.45a
22	8.77a	8.17a
32	7.82a	7.29a
Mean	8.06	7.55

Means with the same letter do not differ in the columns, according to Tukey test ($p \leq 0.05$).

Bioactive compounds and antioxidant activity

There were no statistically significant differences ($p > 0.05$) observed in the contents of yellow flavonoids and antioxidant activity during storage period. However, a significant effect of storage time was noted for several other components. Vitamin C exhibited a noteworthy impact ($p < 0.05$), with the highest content recorded on the 14th day (23.34 mg per 100 g) and the lowest on the 32nd storage day (17.67 mg per 100 g) (Figure 6A), reflecting a reduction of 17.50% over the storage duration. Total anthocyanins (ANT) also demonstrated a significant effect due to storage time ($p < 0.05$), resulting in variations in their content throughout the storage period. Total extractable polyphenols showed a notable impact ($p < 0.01$), suggesting fluctuations in their content during the storage period.

Betaxanthins were influenced by both storage time and the type of extract ($p < 0.01$), highlighting the intricate relationship between these factors and their effects on betaxanthin content. Furthermore, a significant interaction was observed between the type of extract and time for betacyanins ($p < 0.01$), underscoring the complex interplay between these variables and their influence on betacyanin content.

The decline in vitamin C content during storage, influenced by temperature and time, is likely linked to alterations in the fruit's surrounding atmosphere. Specifically, it can be attributed to factors such as oxygen exposure and fruit water loss. These changes may be mitigated by restricting gas exchange, which, in turn, inhibits the oxidation of this antioxidant (Brunini and Cardoso, 2011; Carvalho et al., 2016). Studies suggest that vitamin C content can exhibit various trends during storage, including increases, decreases, or remaining relatively constant. This behavior is akin to the observations made by Brunini and Cardoso (2011) in the case of *H. undatus*. They noted a pattern of initial increase in vitamin C content, followed by a slight decrease towards the end of the storage period. The highest recorded content was 30.18 mg per 100 g, while the lowest was 26.41 mg per 100 g during the storage process. It's noteworthy that compared to many other fruits, pitayas, particularly red varieties, exhibit relatively high content of vitamin C. While most cetaceans are typically low in vitamin C, with content not exceeding 1.1 mg per 100 g, research by Esquivel et al. (2007) evaluating different *Hylocereus* pitaya genotypes found vitamin C content ranging from 26 to 58 mg per 100 g. Among these, red-fleshed pitayas stood out, demonstrating their superiority in this regard (Choo and Yong, 2011; Abreu et al., 2012).

The anthocyanin content in pitaya exhibited fluctuations during storage, showing an increase up to the 14th day (14.14 mg 100 per g) and a subsequent decline to 11.02 mg per 100 g by the 32nd day (Figure 6B). This initial increment until the 14th day likely contributed to the higher color saturation observed in the fruit peel during the same storage period (Figure 4A) and may have played a role in intensifying pulp color saturation over time (Figure 4B). It's worth noting that, while pitayas have a red-purple color suggesting the presence of high anthocyanin content, this study's findings indicate lower anthocyanin levels compared to other fruits like açaí (21.23 mg 100 per g) and strawberries (21.69 mg per 100 g) (Teixeira et al., 2008), and West Indian cherry (18.90 mg 100 per g) (Rufino et al., 2010). Pitaya research suggests that its coloring is primarily attributed to betacyanins (García-Cruz et al., 2016; Fathordoobady et al., 2020) and the absence of anthocyanins is associated with the presence of betacyanins (Wu et al., 2006). These compounds typically do not coexist within the same species (Hua et al., 2018).

The betacyanin content varied significantly among different extract types (Table 2) and exhibited changes with storage time (Figure 6C). Alcohol extraction yielded a higher pigment content (70.31 mg per 100 g), which was 11.26% higher than water extraction. Concerning the effect of storage time, the pitayas had an average betacyanin content of 73.35 mg per 100 g at harvest, which decreased to 57.91 mg per 100 g at the end of storage, resulting in a 21.05% reduction in betacyanin content (Figure 6C).

A similar trend was observed for betaxanthins concerning extract types (Table 2) and storage time (Figure 6D), with a 12.83% decrease. Interestingly, the values of betaxanthins exceeded those of betacyanins, consistent with findings reported by García-Cruz et al. (2013). Priatni and Pradita (2015) also noted higher betacyanin extraction with a methanol solution compared to water. These pitayas exhibit higher betalain pigment content compared to other cacti fruits (Gárci-Cruz et al., 2013), which are vegetable pigments commonly used in the food industry as

functional food colorants (Winson et al., 2020).

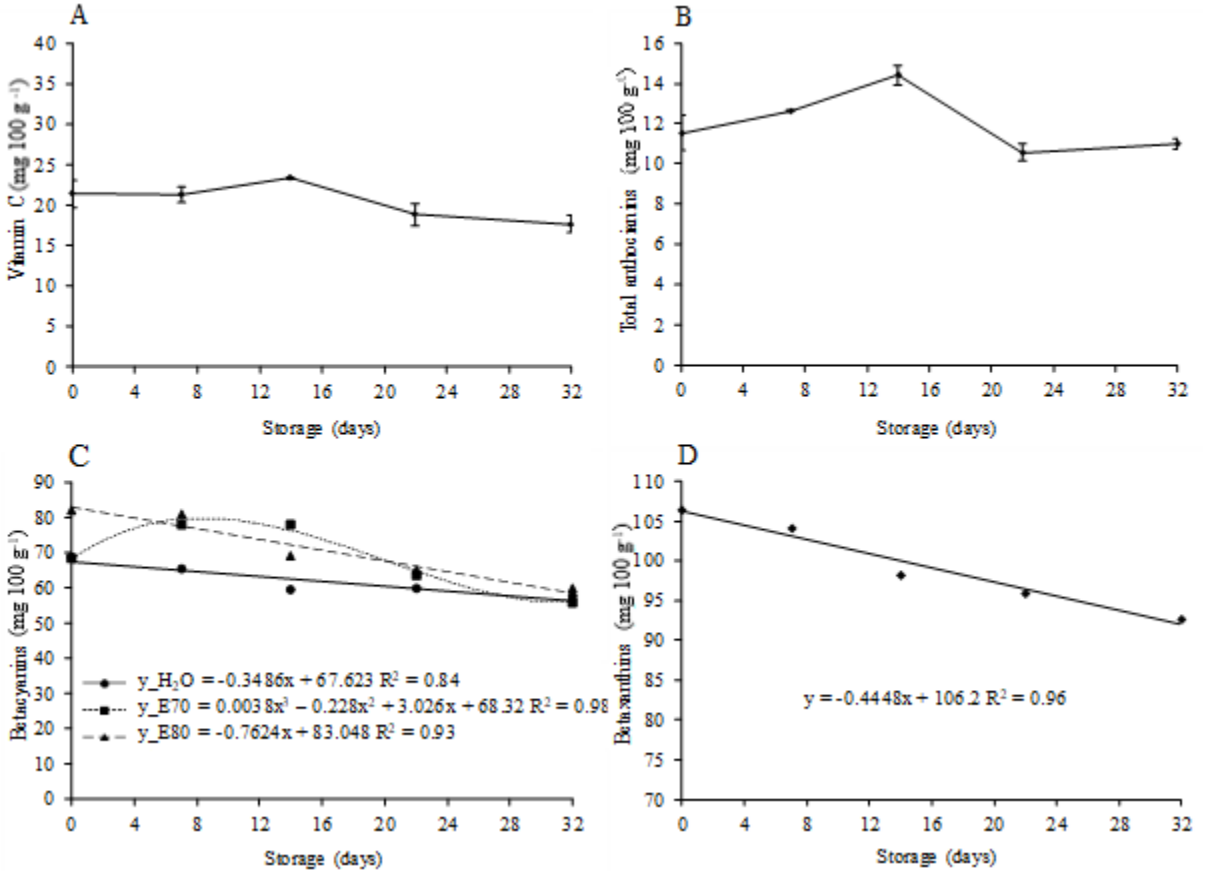

Figure 6. Vitamin C (A), total anthocianins (B), betacyanins in different extracts (C), and betaxanthins (D) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity). y_H₂O – water; y_E70 – ethanol 70% (ethanol:water 70:30); and y_E80 – ethanol 80% (ethanol:water 80:20).

Table 2. Betacyanins and betaxanthins of pitaya (*Hylocereus polyrhizus*) extracted with water and ethanol at 70 and 80%.

Extractor	Betacyanins (mg per 100 g)	Betaxanthins (mg per 100 g)
H_2O	62.39b	90.16c
Ethanol 70%	69.00a	97.88b
Ethanol 80%	71.61a	110.54a
Mean	67.67	99.53

Means with the same letter do not differ in the columns, according to Tukey test ($p \leq 0.05$). H_2O – water; ethanol 70% – ethanol:water 70:30; ethanol 80% – ethanol:water 80:20.

As highlighted by Thirugnanasambandham and Sivakumar (2017), the dragon fruit processing industry has shown a growing interest in betalains since they have been recognized as natural antioxidants with potential positive health benefits for humans. Additionally, the utilization of betalains may contribute to the sustainable development of semi-arid regions, which are often underdeveloped, and create opportunities within the market for cacti fruits. Therefore, pitaya (*H. polyrhizus*) stands out not only as a valuable source of betalains but also as a potential alternative source of income for semi-arid regions. This is particularly significant

because pitaya is one of the most water-efficient fruit plant species, making it well-suited for cultivation in these water-scarce areas.

The degradation of betacyanins is influenced by factors such as pH, temperature, and light, with higher levels of these factors leading to increased degradation (Priatni and Pradita, 2015; Sánchez-Chávez et al., 2015). It's worth noting that the betacyanins and betaxanthins contents in this study were higher than those observed by García-Cruz et al. (2013), who found contents ranging from 28.60 to 47.00 mg per 100 g for red and orange pitayas from Tepexi de Rodríguez, Mexico, and were in a similar range to those reported by Vaillant et al. (2005) for various pitaya cultivars in Nicaragua, with contents ranging from 32 to 41 mg per 100 g. Additionally, the values were close to those observed by Castellar et al. (2003) for fruits of different *Opuntia* species from the Murcia region in Spain, with contents of 67.00 to 80.10 mg per 100 g.

The storage conditions did not have a significant impact on the pitaya yellow flavonoids content, which had an overall mean of 7.85 mg per 100 g of pulp (Table 3). In comparison, Lima et al. (2013) reported variations in flavonoid content ranging from 0.88 to 6.03 mg per 100 g in the pulp of various Brazilian native commercial pitaya species, with *H. costaricensis* standing out for its significantly higher yellow flavonoid content compared to other species. It's important to note that red pitaya generally has higher flavonoid content than the white variety, with the fruit peel containing the highest concentration (Kim et al., 2011). Another study reported a flavonoid content of 7.21 mg per 100 g for *H. polyrhizus* fruits grown in Taiwan (Wu et al., 2006).

The total extractable polyphenols (TEP) content showed a significant decrease during storage (Figure 7), reaching its lowest point on the 24th day (19.25 mg per 100 g), representing a reduction of more than half over the storage period (59.41%). Despite this decrease, the pitayas in this study maintained an average TEP content of 29.50 mg per 100 g, which is higher than various genotypes of pitaya, including *S. megalanthus* (12.31 mg per 100 g), *S. setaceus* (15.81 mg per 100 g), *H. undatus* (17.28 mg per 100 g), and *H. costaricensis* (23.15 mg per 100 g) (Lima et al., 2013). The TEP content in this study is also notable when compared to the same species, *H. polyrhizus*, with values of 21.00 mg per 100 g (Lim et al., 2007) and 24.22 mg per 100 g (Choo and Yong, 2011). However, it falls short of the values reported by García-Cruz et al. (2016) for red pitayas (ranging from 53.59 to 70.77 mg per 100 g). The decrease in TEP content during storage can be attributed to various factors, including the consumption of compounds through biochemical processes and degradation (Santos et al., 2016). These differences may also be influenced by environmental variations and/or differences in fruit maturity stage.

Table 3. Mean values of flavonoids and total antioxidant activity (AAT) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

Storage time (days)	Variable	
	Flavonoids (mg per 100 g)	AAT (ABTS) (µmol Trolox per g)
Harvest	7.69a	1.42a
7	9.03a	1.39a
14	8.34a	1.37a
22	7.70a	1.16a
32	6.46a	1.15a
Mean	7.85	1.30

Means with the same letter do not differ in the columns, according to Tukey test ($p \leq 0.05$).

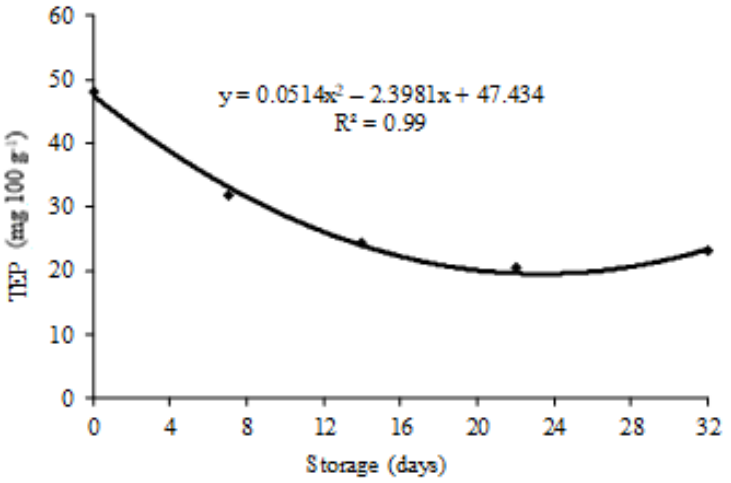

Figure 7. Total extractable polyphenols (TEP) of pitaya fruits (*Hylocereus polyrhizus*) during refrigerated storage (10 ± 1°C and 90 ± 5% relative humidity).

The total antioxidant activity (AAT) was not significantly affected by refrigerated storage, although there was a slight decrease in the mean values (Table 3). Even though there was a decrease in vitamin C, phenolic compounds, flavonoids, betacyanins, and betaxanthins over time, this reduction was not sufficient to cause a decrease in AAT. A similar trend was observed by Obenland et al. (2016) in pitayas stored at 10°C, but there was a slight decrease in AAT when stored at 5°C. Comparing the AAT of pitaya with other commonly consumed fruits, it appears that pitaya has relatively low AAT. However, Abreu et al. (2012) reported that pitaya has a high antioxidant capacity when assessed using the beta carotenoid/linoleic acid method. This high antioxidant capacity may be linked to the high content of betacyanins in red pitaya pulp (Wu et al., 2006). It's possible that the ABTS method used in this study might not be the most suitable for measuring the antioxidant capacity in pitaya, or the specific growth conditions in commercial orchards, where plants are conditioned to low stress, could have influenced its AAT. Further research with different methods for capturing free radicals is needed to comprehensively assess pitaya's antioxidant capacity. Furthermore, pitaya remains a significant source of AAT, with values ranging from moderate to high, making it an important source of phytochemicals (García-Cruz et al., 2013; Song et al., 2016). When combined with other fruits and vegetables, it can contribute to a wide range of health benefits, including antioxidant effects, free radical scavenging, and protection against infections, all of which are advantageous for human health (Winson et al., 2020).

Conclusions

The refrigerated storage of pitaya (*Hylocereus polyrhizus*) at 10 ± 1°C and 90 ± 5% relative humidity proved effective in maintaining the fruit's good appearance, high sugar content, SS/TA ratio, and pulp yield for up to 32 days without a significant loss of quality. During this storage period, the fruit also retained elevated firmness and maintained its betacyanin and betaxanthin content. The antioxidant activity of pitaya is closely related to its anthocyanins, polyphenols, and betacyanins. This research holds significant importance in the agribusiness sector and has the potential to contribute to the agricultural development of the Brazilian semi-arid region. It offers a promising alternative for the distribution of pitaya fruit to both the national and international markets, as these fruits are rich

sources of bioactive compounds and other health-beneficial nutrients.

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