



Effect of post-harvest storage on chemical composition and deterioration of cactus cladodes [*Opuntia ficus-indica* (L.) Mill and *Nopalea cochenillifera* Salm-Dyck]

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[†] In Memoriam

ABSTRACT: The daily cut-and-carry system is commonly used for cactus harvest, which increases manpower costs. This research aimed to determine the effect of the post-harvest storage period (up to 56 days post-harvest) on the chemical composition and anatomical traits of cactus cladodes [*Opuntia ficus-indica* (L.) Mill cv. 'IPA 20' and *Nopalea cochenillifera* Salm-Dyck cv. 'Miúda']. Treatments included two cactus cultivars ('IPA 20' and 'Miúda') and post-harvest storage periods (0, 8, 16, 24, 32, 40, 48, and 56 days). The experimental arrangement was split-plot in a completely randomized design with eight replications. Dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) from cladodes were analyzed by order (primary, secondary, and tertiary) at the end of each storage period. Deterioration rate and root emergence were evaluated using a rank scale (1 to 5). Cladode storage up to 56 days did not reduce cactus CP and NDF, conform cactus cladodes; however, WSC reduced from 281 to 235 g kg⁻¹ (at 0 days of storage) and from 521 to 475 g kg⁻¹ (at 56 days of storage) for 'IPA 20' and 'Miúda' cultivars, respectively. Low variation occurred for DM and OM concentrations, however 'Miúda' had greater deterioration during the storage period than 'IPA 20', with ranks of 4.7 and 3.2, respectively, at the 56 days of storage. There was a linear response regarded the deterioration effect for 'IPA-20' cultivar and a quadratic response for cultivar 'Miúda'. Both species presented a reduction in epidermal area and an increase in parenchymal area after 32 days of storage. The 'IPA 20' cultivar may be stored up to 56 days with less nutrient loss. The 'Miúda' cultivar, presented greater deterioration, must be stored for a post-harvest period of up to 32 days.

Key words: anatomy; cactaceae; conservation; cactus forage; semiarid

Efeito do armazenamento pós-colheita na composição química e deterioração de cladódios de palma [*Opuntia ficus-indica* (L.) Mill e *Nopalea cochenillifera* Salm-Dyck]

RESUMO: Um sistema diário de corte é comumente usado para a colheita da palma, o que aumenta os custos de transporte. Esta pesquisa teve como objetivo determinar o efeito de um período de armazenamento pós-colheita (até 56 dias pós-colheita) na composição química e características anatômicas de cladódios de palma [*Opuntia ficus-indica* (L.) Mill cv. IPA 20 e *Nopalea cochenillifera* Salm-Dyck cv. Miúda]. Os tratamentos foram duas cultivares de palma (IPA 20 e Miúda) e períodos de armazenamento pós-colheita (0, 8, 16, 24, 32, 40, 48 e 56 dias). O arranjo experimental foi parcelas subdivididas em delineamento inteiramente casualizado com oito repetições. A matéria seca (MS), matéria orgânica (MO), a proteína bruta (PB) e a fibra em detergente neutro (FDN) do cladódio foram avaliadas por ordem (primária, secundária e terciária) ao final de cada período de armazenamento. A taxa de deterioração e emergência de raízes foram avaliadas utilizando escala de classificação (1 a 5). O armazenamento dos cladódios por até 56 dias não reduziu a PB e a FDN das palmas; entretanto, os CHO variaram de 281 a 235 g kg⁻¹ (0 dia de armazenamento) e de 521 a 475 g kg⁻¹ (56 dias de armazenamento), para IPA 20 e Miúda, respectivamente. Pouca variação ocorreu nas concentrações de MS e MO. A cultivar Miúda apresentou maior deterioração durante o período de armazenamento que a IPA 20, com classificações de 4,7 e 3,2 aos 56 dias de armazenamento, respectivamente. Houve resposta linear em relação ao efeito de deterioração para a cultivar IPA-20 e resposta quadrática para a cultivar Miúda. Ambas as espécies apresentaram redução da área epidérmica e aumento da área parenquimatosa após 32 dias de armazenamento. A palma IPA 20 pode ser armazenada por até 56 dias com menor perda de nutrientes. A cultivar Miúda, apresentou maior deterioração, devendo ser armazenada por período de pós-colheita, de até 32 dias.

Palavras-chave: anatomia; cactáceas; conservação; palma forrageira; semiárido



Introduction

Forage cactus represents an important fodder resource for ruminants in the dry regions of the world (Herrera-Angulo et al., 2023), because of the remarkable morphological and physiological characteristics, such as great water storage capacity in stem, modified leaves, presence of spines, thick and waxy cuticle, green epidermis, reduced stomatal conductance and few stomata, and crassulacean acid metabolism (CAM) (Lenzi & Orth, 2012), that increase their plant adaptation to semiarid regions.

Brazil has largest cactus cultivation in the world. The forage cactus is a culture adapted to climatic conditions of the Brazilian semiarid region (Dubeux Jr. et al., 2022b), characterized by seasonal forage production typically occurs for up to 4 months during rainy season (Dubeux Jr. et al., 2010). The two major cultivated cactus genera in Brazilian semiarid are *Opuntia* and *Nopalea* (Santos et al., 2022a). Due to the seasonal forage production, in many cases, livestock producers are forced to import grains from other regions to reach the animal nutrient requirements, representing increase of production costs (Conceição et al., 2018).

Cactus chemical composition varies conform cultivars, cladode age, crop management, and season (Dubeux Jr. et al., 2021). Forage cactus has high water and non-fibrous carbohydrates (NFC) contents, as well as low concentrations of CP, NDF and ADF (Paula et al., 2019), and a fast passage rate, which can compromise ruminal functions when is supplied in inadequate quantities (Lopes et al., 2020). Regardless of cladode age and order, they are highly digestible (Pessoa et al., 2020). On the other hand, limited information is available regarding the anatomical characteristics of the cactus, especially concerning the distribution and composition of its various tissues. These tissues play a role in certain cactus varieties and understanding them could provide insights into potential correlations with quality (Gonçalves et al., 2022). In addition to feed shortage, water scarcity compromises livestock performances in dry areas. Because of its succulence, cactus could also overcome this constraint (Ben Salem, 2009).

Cactus is usually harvested using manual cut-and-carry practices. After harvesting, cactus is transported daily from the field to the feeding site, resulting in increased feeding costs due to labor and transportation expenses (Santos et al., 2022b). Post-harvest storage of cactus cladodes under shade and protected from weathering might reduce feeding costs, representing an important technology (Figure 1) to increase the efficiency of utilization of this important fodder. The forage cactus is a CAM metabolism plant, which when harvested has its stomata closed, these being internal and deep, in addition to having a thick cuticle. Santos et al. (1992) evaluated post-harvest storage of cactus cladodes for up to 16 days, and reported no significant losses in DM, CP, crude fiber (CF), and WSC for three cactus cultivars ('Redonda', 'Gigante', and 'Miúda'). In another trial, Santos et al. (1998) observed that cactus stored up to 16 days did not affect the milk yield of lactating dairy cows. Carvalho et



Figure 1. Representative scheme of harvesting, transport and post-harvest storage of forage cactus

al. (2020) found that post-harvest storage for up to 60 days of *N. cochenillifera* ('Doce' and 'Baiana' genotypes) and *O. stricta* ('Orelha de elefante mexicana') did not compromise the chemical composition.

Storage periods (> 16 days) for cactus cladodes may further reduce operation costs; however, it is not known which effect longer-term storage will have on the rate of deterioration, anatomy, and integrity of cactus cladodes. Based on these considerations, the hypothesis is that post-harvest storage of different cactus cultivars can affect the anatomical characteristics and chemical composition of the stored cladodes.

The objective of this research was to evaluate storage periods up to 56 days and their effects on the chemical composition, histological characteristics, and rate of deterioration of stored cactus cladodes of different cultivars.

Materials and Methods

Site and experimental material

The experiment was carried out at the Experimental Station of the Agronomic Institute of Pernambuco, located in Caruaru, PE, Brazil. The geographical coordinates were 08°14'18" S and 35°55'20" W, with an elevation of 537 m above sea level. The average annual rainfall was 727 mm.

The establishment of the cactus cultivars *Opuntia ficus-indica* (L.) Mill cv. 'IPA 20' and *Nopalea cochenillifera* Salm-Dyck cv. 'Miúda' followed the planting and management recommendations for the crop according to Santos et al. (2022b). At the time of planting, 20 Mg ha⁻¹ (Dubeux Jr. et al., 2022a) of cattle manure was applied in dressing. The harvest occurred two years after establishment. Mother cladodes (i.e., cladodes used for establishment) were not harvested. The harvested portion was not chopped, and branches were kept together. From each field, 1,600 kg of green matter of cladodes were harvested for both species. This material was spread in 200-kg piles under a shed with a cement floor and roofed with fiber cement; the shed was opened laterally.

Air temperature and rainfall

The average rainfall recorded during the forage cactus growing period was 782.6 mm, above the average annual variation of 694 mm, being average temperature of 23.3 °C was recorded.

Air temperature under the shed and outside, as well as the relative humidity, was recorded daily during the experimental period using a digital thermo-hygrometer Incoterm®. Internal and external temperature, air relative humidity, and monthly rainfall averaged 28.6 ± 1.1 °C; 28.4 ± 0.9 °C; $51.5 \pm 5.7\%$, and 10.7 ± 6.4 mm, respectively. Monthly total rainfall during the experimental period was 1.1, 17.7, and 13.3 mm for the storage period, according to Agronomic Institute of Pernambuco.

Experimental treatments

Experimental treatments included two cactus cultivars ('IPA 20' and 'Miúda') and different post-harvest storage periods (0, 8, 16, 24, 32, 40, 48, and 56 days). Cladode order (primary, secondary, and tertiary) was also a treatment factor.

Evaluations

Visual observations were performed to evaluate cladode deterioration and cladode root emission. Observations were made for each pile at the end of each post-harvest storage period. The rank scale ranged from 1 to 5, where rank 1 means no cladode deterioration and no root emission; rank 2 means at least one cladode was deteriorated and presented root emission; rank 3 means less than 25% of cladodes presented root emission and deterioration; rank 4 between 25% and 50% of cladodes presented root emission and deterioration; rank 5 means more than 50% of cladodes presented root emission and deterioration.

At each post-harvest time, cladodes were sampled by order (primary, secondary and tertiary) to determine the DM (Method 930.15), ash (Method 942.05), crude protein (CP) (Method 984.13), and water-soluble carbohydrate (Method 999.03) levels according to [AOAC \(2005\)](#). Analysis of neutral detergent fiber (NDF) were performed according to [Van Soest et al. \(1991\)](#).

Two disk-shaped samples (3 × 3 cm) were collected from the center of the secondary cladode for anatomical measurements. All these samples were immediately immersed and fixed in formalin-aceto-alcohol (FAA) (90 mL ethanol 50%, 5 mL glacial acetic acid, and 5 mL formaldehyde) for 48 h, and sent to the Forage Anatomy Laboratory at the Animal Science Department, UFRPE (Federal Rural University of Pernambuco). After fixation, samples were immersed in 70% ethanol solution for histological studies ([Sass, 1958](#)). Histological sections were made manually using a microtome lamina. Samples were immersed in 6% solution of sodium hypochlorite for five minutes to avoid precipitation between cell content and the dye. Samples were thoroughly washed three consecutive times with deionized water for 15 minutes ([Johansen, 1940](#)).

After the initial procedures, the histological sections were treated following the procedure of [Tolivia & Tolivia \(1987\)](#). Slides and microslides were cleaned with ethanol and washed with the vegetal tissue fixer in slide named Polly-L-Lysine, 9:1 diluted in deionized water (9 ml deionized water:

1 mL Polly-L-Lysine) thereafter. Microslides were fitted with Entelan®. Epidermal and parenchymal areas were measured in the histological sections. Measurements were performed using an OLYMPUS BX41 microscope with a coupled digital camera and SIGMA SCAN PRO 5 software.

Experimental design and statistical analyses

For DM, CP, NDF and ash, was applied a split-split plot arrangement in a complete randomized design with eight replications as described by the [Equation 1](#):

$$Y_{ijkl} = \mu + C_i + E_{ij} + P_k + (C \cdot P)_{ik} + e_{ijk} + O_l + (C \cdot O)_{il} + (P \cdot O)_{kl} + (C \cdot P \cdot O)_{ikl} + \varepsilon_{ijkl} \quad (1)$$

where: Y_{ijkl} represents the average response of cultivar i , replication j , storage period k , and cladode order l . Cultivar (C_i), storage period (P_k), and cladode order (O_l) as well as their interactions [($C \cdot P$) $_{ik}$, ($C \cdot O$) $_{il}$, ($P \cdot O$) $_{kl}$ and ($C \cdot P \cdot O$) $_{ikl}$] were considered fixed effects. Storage periods (P_k) were treated as repeated measures, since cladode samples were collected from the same pile at different time intervals. Random effects included main plot error (E_{ij}), split-plot error (e_{ijk}), and split-split plot error (ε_{ijkl}).

For WSC, rate of deterioration, and cladode rooting, was applied a split-plot arrangement in a complete randomized design was used. The main plot included the cultivar and the split-plot included the storage periods, as described by the [Equation 2](#):

$$Y_{ijkm} = \mu + C_i + E_{ij} + P_k + (C \cdot P)_{ik} + e_{ijk} \quad (2)$$

where: the descriptions of its terms are the same as those described in [Equation 1](#).

Statistical analyses were performed by the PROC MIXED procedure ([Littell et al., 1996](#)) from SAS ([SAS, 2003](#)). Means for different cultivars, storage periods, and cladode orders and their interactions were compared by the F-test and least square means (LSMEANS). The LSMEANS were compared by the PDIFF procedure adjusted by Tukey mean test. Orthogonal contrasts were also performed for different storage periods. All differences were considered significant at 5% probability.

Results and Discussion

Visual aspects

Cactus cultivars and post-harvest storage period interacted ($p < 0.05$) for the level of deterioration and root emission from cladodes (Table 1 and 2). The 'Miúda' cultivar presented a greater level of deterioration (up to 4.7) than 'IPA-20' cultivar (up to 3.2) during most of the storage periods. 'Miúda' cultivar can be stored up to 32 days (Table 1). Greater grades were assigned at the final time points (40, 48, and 56 days), with averages of 3.6, 4.0, and 4.7 respectively (Table 1).

Table 1. Ranking scale[§] of cladode deterioration of different forage cactus cultivars, after varying lengths of post-harvest storage

Post-harvest storage period (days)	Deterioration	
	'IPA-20'	'Miúda'
0	1.0dA	1.0eA
8	1.1bB	3.0dA
16	2.2cB	3.0dA
24	2.7bA	3.0dA
32	3.0abA	3.2dA
40	3.0abB	3.6cA
48	3.0abB	4.0bA
56	3.2aB	4.7aA
<i>p</i> value		
Period (SP)	0.0001	0.0001
Cultivars (C)	0.0002	0.0001
SP × C	0.0002	0.0001
Linear effect	0.0005	0.0001
Quadratic effect	0.4475	0.0001
Standard error	0.2215	

[§]Ranking scale ranging from 1 to 5, as described: 1. no cladode deterioration was observed; 2. at least one cladode was deteriorated; 3. less than 25% of cladodes presented deterioration; 4. between 25% and 50% of cladodes presented deterioration; 5. more than 50% of cladodes presented deterioration.

[¶]Means followed by the same letter (lowercase letters across columns and capital letters across rows) do not differ significantly by F test ($p > 0.05$).

Table 2. Ranking scale[§] of root emission from cladodes of different forage cactus cultivars, after varying lengths of post-harvest storage

Post-harvest storage period (days)	Root emission	
	'IPA-20'	'Miúda'
0	1.0bA	1.0aA
8	1.0bA	1.0aA
16	1.0bA	1.0aA
24	1.0bA	1.0aA
32	1.0bA	1.0aA
40	1.0bA	1.0aA
48	4.4aA	1.0aB
56	4.5aA	1.0aB
<i>p</i> value		
Period (SP)	0.0023	0.0001
Cultivars (C)	0.0010	0.0001
SP × C	0.0011	0.0001
Linear effect	0.6328	-
Quadratic effect	0.7203	-
Standard error	0.2011	

[§]Ranking scale ranging from 1 to 5, as described: 1. no root emission was observed; 2. at least one cladode showed root emission; 3. less than 25% of cladodes showed root emission; 4. between 25% and 50% of cladodes showed root emission; 5. more than 50% of cladodes showed root emissions.

[¶]Means followed by the same letter (lowercase letters in the columns and capital letters in the lines) do not differ significantly by the F test ($p > 0.05$).

The 'Miúda' cultivar did not present root emission from cladodes during the studied period; however, it did show some young cladodes emerging from the old ones (Table 1). 'IPA-20' cultivar developed linear response by orthogonal contrast, while 'Miúda' cultivar had a quadratic response for cladode deterioration with increasing length of storage periods. For root emission, there was no linear or quadratic response for 'IPA-20' cultivar. 'Miúda' cultivar had no root emission during the storage periods (Table 2).

The grades attributed to cladode deterioration indicated that 'Miúda' had greater deterioration than 'IPA-20', 32 days after storage (Table 1). Thus, storage periods for 'Miúda' should be shorter than 'IPA-20'. In addition, during the storage period the cladodes of 'Miúda' cultivar had greater presence of insects, such as ants, which can have contributed to greater cladode deterioration. In the literature, only a few papers have described likely explanations for cladode deterioration during post-harvest storage. Cantwell (2001) observed that after cladode harvest with approximately 15 to 20 cm, heat generation occurred in the center of the pile, this contributed to cladode deterioration and favored proliferation of microorganisms that caused rot. The cactus pear is a food with high water content; thus, it can easily suffer microbiological contamination (Vieira et al., 2022). The greater WSC concentration of 'Miúda' (Santos et al., 1992) may explain the greater deterioration of 'Miúda' cladodes.

During the morning, at harvesting time, cactus plants produce sugars from malic acid synthesized at previous night, via CAM photosynthetic metabolism (Black & Osmond, 2003). Greater deterioration observed for 'Miúda' (*Nopalea*) cultivar can also be associated with greater carbohydrate concentration compared with *Opuntia* cultivars. During the transformation of malic acid into glucose, the acidity decreases, causing an elevation in the pH of the cladodes as the day advances (Keeley, 1999). Furthermore, Silva et al. (2017) reported variations in pH levels between *Nopalea* and *Opuntia* cactus harvested in the morning. Notably, the cactus *Opuntia* (4.59) exhibited a significantly lower pH compared to *Nopalea* (5.01). It is worth mentioning that larger cladodes such as 'IPA-20' can result in greater aeration within the piles, unlike piles with 'Miúda'. Based on this, there may have been a greater contact surface between the cladodes of the 'Miúda' forage cactus, which may have contributed to greater humidity between the cladodes and consequently greater deterioration (Table 1).

Another aspect to consider is the quantity of stomata present in *Opuntia* and *N. cochenillifera*. *Opuntia* typically has 10 to 30 stomata per mm² of cladode surface, while *N. cochenillifera* has approximately 50 per mm² of cladode surface (De La Barrera & Nobel, 2004). This difference may contribute to a higher rate of deterioration observed in the cladodes of the 'Miúda' forage cactus.

Visual observations indicated that only 'IPA-20' cladodes emitted roots (Table 2). The cactus fields were established at the same time; thus, cactus plants were approximately the same age. The absence of root emission from 'Miúda' may be related to greater levels of cladode deterioration (Table 2). Although 'Miúda' forage cactus has a lower moisture content, this can vary depending on the species, storage conditions, time of year and harvest age.

'Miúda' exhibited a higher number of young cladodes compared to 'IPA-20'. This is likely to result in reduced root emission from 'Miúda' cladodes in comparison to 'IPA-20' (Table 2). The hormonal balance for plant part, nutritional status, and environmental conditions of storage

are preponderant to regulate the development of the root emission process in cactus cladodes (Snyman et al., 2006). Under cultivation conditions, few papers have reported root emission by stored cladodes. Lopes et al. (2009) evaluated different pre-planting shade exposure treatment of cladodes (0, 8, 16, 24, and 32 days) and planting period after shade exposure (5, 10, 15, 20, and 25 days). The authors observed increased root emission by *Opuntia ficus-indica* Mill. cv. 'Gigante' when cladodes were exposed to 32 days of shade and planted between 15 and 20 days after this treatment. Thus, cladodes were planted 47 days after harvest. This period is similar to the current experiment, where 'IPA-20' cladodes also emitted roots after 48 days of post-harvest storage.

Chemical composition

Significant interaction ($p < 0.05$) occurred for cactus cultivars, cladode order, and post-harvest storage period for DM concentration of stored cladodes (Table 3).

These variations, however, were slight. The DM concentration for the 'IPA-20' ranged from 87 to 118 g kg⁻¹ for secondary cladodes stored for 16 days and primary cladodes with 40 days of storage, respectively (Table 3). The DM concentration in the 'Miúda' clone ranged from 141 to 196 g kg⁻¹ at 16 and 48 days of storage on the tertiary and primary cladodes, respectively (Table 3). Variations in the DM content of cladodes may be related to water loss caused by plant evapotranspiration during the storage period (Carvalho et al., 2021). There was no linear or quadratic effect by orthogonal contrast analysis for the cultivar 'Miúda' and DM concentration, regardless cladode order. 'IPA-20' cultivar had quadratic effect on DM concentration as a function of storage periods for all cladode orders. The results may also be related to the formation of piles with

non-uniform cladodes, and the consequence of cladode overlap promoted by storage. In addition to the variation in the number of cladodes by common order in cactus (Santos et al., 1990a).

Different DM concentration of stored cladodes from two cultivars (Table 3). In the CAM mechanism, plants under drought stress close their stomata during the day to reduce water loss to the environment (Flores-Hernández et al., 2004). This result is similar to the ones obtained by Santos et al. (1992), working in São Bento do Una in the Agreste region of Pernambuco, who observed small DM variations for cactus cultivars 'Redonda', 'Gigante', and 'Miúda' stored up to 16 days post-harvest. Silva et al. (2017) observed significant DM variations only at day 0 (127 g kg⁻¹) and day 7 (118 g kg⁻¹) of storage and had no significant DM difference until 21 days of storage (116 g kg⁻¹) to *Opuntia stricta*. In general, *Nopalea cochenillifera* cv 'Miúda' has greater dry matter concentration than 'IPA-20' (Cardoso et al., 2019), regardless of storage period (Santos et al., 1990b), which is in accordance with the results obtained in the present experiment.

In addition to physiological mechanisms, anatomical adaptations also contribute to small variations in DM concentration. A thick cuticle with trichomes and large stomata in low density with a deep substomatic chamber (Perrotta & Arambarri, 2018) likely contribute to reduce water loss from cladodes to the environment. Regarding differences between species, cactus of the genus *Opuntia* have a thick cell wall, due to greater lignification of the secondary cell wall (Silva et al., 2010). The quadratic effect on DM concentration observed for the cultivar 'IPA-20' considering all cladode orders is likely associated with an improved mechanism to water retention rather than 'Miúda'. However, due to great water content, forage cactus

Table 3. Dry matter concentration (g kg⁻¹) of forage cactus cladodes of two cultivars according to cladode order and storage periods

Post-harvest storage period (days)	'IPA-20'			'Miúda'		
	Cladode order					
	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
0	111abA	109aA	100abA	171bcB	186aA	170aB
8	114abA	96abB	97abB	183bA	172abB	169aB
16	100bA	87bA	88bA	164cA	168bA	141cB
24	102bA	97abA	91bA	193abcA	179abAB	169aB
32	108abA	100abA	102abA	206aA	174abB	147bcC
40	118aA	101abB	108aAB	194abA	178abB	144cC
48	107abA	95abA	109aA	196abA	180abB	151bC
56	106abA	98abA	110aA	194abcA	185aA	162abB
<i>p</i> value						
Cultivars (C)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Period (SP)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Order (O)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
C × SP × O	0.0002	0.0124	0.0135	0.0003	0.0277	0.0001
Linear effect	0.0429	0.0089	0.0055	0.9489	0.0887	0.5833
Quadratic effect	0.0398	0.0171	0.0040	0.4067	0.1579	0.8027
Standard error	2.2215					

*Means followed by the same lowercase letters in the columns do not differ from each other using the Tukey test ($p > 0.05$) and means followed by the same uppercase letters from the rows, purchasing within each cultivar, do not differ from each other using the Tukey test ($p > 0.05$).

represents an excellent water source for animals, especially in semiarid regions, as well as with reduction in voluntary of water intake in diets containing forage cactus (Cardoso et al., 2019).

Storage period did not affect ($p > 0.05$) the CP and NDF levels of the tested cactus cultivars. There was a significant interaction between cultivar and cladode order for CP and NDF concentrations (Table 4).

Cactus CP did not vary during the storage period for the different cactus species (Table 4). Greater cladode CP concentration ($p < 0.05$) was observed in the secondary order for 'IPA-20'; no differences were observed between primary and tertiary cladode order for this cultivar. 'Miúda' presented greater CP concentration in the tertiary cladodes; no differences were observed between primary and secondary cladodes (Table 4). In research evaluating the chemical composition of different stages of maturity of cladodes, it was observed that there was no variation in the CP content depending on the maturity of the cladodes, with an average of 41.7 g CP/kg DM, on the other hand there was a tendency to increase of NDF with increasing maturity (Naorem et al., 2022). Variations in the chemical composition of 'Miúda' cultivar can be related to its greater growth speed, presenting a greater number of cladodes, in a shorter cultivation time (Silva, 2019).

Greater NDF concentration was observed for 'IPA-20' than 'Miúda' (Table 4). Regardless of cactus species, primary cladodes presented greater NDF concentration than younger cladodes. NDF levels are in accordance with the levels reported in the literature (Santos et al., 2019). It is worth highlighting, the forage cactus has low levels of effective fiber, which is important for animal diets (Gama et al., 2020).

Cactus may be stored for up to 56 days after harvesting without a decrease in CP. Cactus CP did vary with cladode order (Dubeux Jr. et al., 2021). The cactus 'IPA 20' presented more CP in the secondary cladode, with no differences observed between the primary and tertiary cladodes. The primary cladodes were the oldest, which likely accounted for the CP reduction compared to secondary cladodes.

In general, the cactus CP observed in this research for 'IPA-20' are similar to the ones reported by Pessoa et al. (2020), who observed an ranging from 42,6 to 48,0 g CP/

kg DM for this clone in cladodes of phenological phases (young, intermediate and mature phase). Batista et al. (2009) observed average ranging from 34 to 44 g CP/kg DM in *Opuntia* cladodes. The results reported in this study for 'Miúda' are similar to those reported by Paula et al. (2019) for the 'Miúda' cultivar (36 g CP/kg DM).

Abidi et al. (2009) observed that cactus CP of *Opuntia* vary with the season, ranging from 30 g kg⁻¹ DM in the summer to 58 g kg⁻¹ DM in the winter. Consequently, the CP concentration in stored cladodes is likely to vary based on the season and time of harvest, should be considered that in the present work the forage cactus harvest season occurred in the month of november, corresponding to the spring season. In general, cactus CP observed for 'IPA-20' and 'Miúda' are greater than the ones observed by Santos et al. (1992), who reported values ranging from 21 to 41 g kg⁻¹ DM after 16 days of post-harvest storage for 'Miúda' and 'Gigante', respectively. The variation in cactus cladode CP for the duration of the storage period is an important indicator of the use of stored cladodes for animal feeding, however, other characteristics as water carbohydrates and deterioration should be considered. Moreover, this practice post-harvest storage has potential for reducing labor and transportation costs.

Besides CP, NDF concentration did not change during the storage periods (Table 4), indicating that 56 days of post-harvest storage were not enough to compromise fiber content of forage cactus, which is an important aspect for stored cactus, and considering that this component is essential for an adequate rumen maintenance. Greater NDF values, however, were observed for 'IPA-20' than for 'Miúda'. Pessoa et al. (2020) observed cactus NDF (268 g kg⁻¹ DM) similar to the ones observed in the current research for 'IPA-20'. In contrast, Gomes et al. (2018) observed average values of 294 g NDF/kg DM for *Opuntia ficus-indica* Mill at one year of growth. Regardless of cactus species, first-order cladodes presented greater NDF values. These cladodes are more lignified, mature, and thicker, with structural functions for the cactus plant. Sáenz et al. (2010) reported that forage cactus cladode age, cladode size, and climatic conditions affect cladode chemical composition and physical structure, increasing fiber concentration with maturity. Fiber has

Table 4. Crude Protein (CP) and Neutral Detergent Fiber (NDF) concentration (g kg⁻¹ DM) of forage cactus cladodes according to cladode order

Cultivar	CP			NDF		
	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
'IPA – 20'	54aB	62aA	57aB	296aA	242aB	232aB
'Miúda'	34bB	35bB	46bA	205bA	190bB	167bC
<i>p value</i>						
Cultivars (C)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Order (O)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
C × O	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Standard error		0.2823			1.4218	

*Means followed by the same lowercase letters in the columns do not differ from each other using the F test ($p > 0.05$) and means followed by the same capital letters in the rows do not differ from each other using the Tukey test ($p > 0.05$).

physiological functions in cactus (López-Palacios et al., 2012), protecting them against harsh environmental conditions.

In contrast to CP and NDF concentrations, which did not vary during the storage period, the WSC concentration decreased. This reduction may be associated with plant respiration during the days of storage. Considering that cacti cladodes present different respiration rates according to temperatures throughout the day (Sampaio et al., 2022). Also reported that respiration rate decreased during the first days of storage, but rapidly reached a steady state, up to 21 days of storage.

Interaction occurred ($p < 0.05$) between post-harvest storage periods and cultivars for cactus WSC (Table 5). Both

Table 5. Water-soluble carbohydrates (WSC) in g kg^{-1} DM in different forage cactus cultivars for different post-harvest storage periods

Post-harvest storage period (days)	Cultivars	
	'IPA-20'	'Miúda'
0	281aB	521aA
8	282aB	520aA
16	276aB	519aA
24	273aB	519aA
32	259bB	514aA
40	251bcB	487bA
48	245cB	485bA
56	235cB	475cA
<i>p value</i>		
Period (SP)	0.0003	0.0001
Cultivars (C)	0.0001	0.0001
SP × C	0.0019	0.0003
Linear effect	0.2665	0.1231
Quadratic effect	0.2554	0.1086
Standard error	0.3544	

CV clone = 12.8%; CV period = 15.6%

*Means followed by the same lowercase letters in the columns do not differ from each other using the Tukey test ($p > 0.05$) and means followed by the same capital letters in the rows do not differ from each other using the F test ($p > 0.05$).

'IPA-20' and 'Miúda' reduced WSC with increasing post-harvest storage period. Regardless of storage period, 'Miúda' presented greater WSC than 'IPA-20'.

During the experimental period, the internal and external temperatures in the shed had variability, ranging from 26.1 to 35.8 °C and 26 to 31.1 °C, respectively. These oscillations likely affected the respiration of stored cladodes and increased WSC use, leading to a decrease in WSC as the storage period increased. Silva et al. (2017) observed that total carbohydrate concentration decreased linearly during storage of erect prickly pear (*Opuntia stricta*), ranging from 870 to 827 g kg^{-1} at 0 and 21 days, respectively, under storage conditions in a covered and ventilated warehouse, and stacked on a wooden platform.

'Miúda' presented greater WSC concentration than 'IPA-20' regarding the different storage periods. This explains why this cultivar is known as 'sweet cactus' by local farmers. Santos et al. (1992) evaluated cactus cladode storage for up to 16 days, and observed an average of 579 g WSC/kg DM for 'Miúda' and 295 g WSC/kg DM for *Opuntia ficus-indica* Mill. cv. 'Gigante' with two years of regrowth. Furthermore, the authors also observed reduction in WSC concentration with longer storage periods.

There was a quadratic effect on ash concentration solely for secondary cladodes of cultivar 'IPA-20'. For cultivar 'Miúda', there was no linear or quadratic effect on ash concentration regarding storage periods for all cladode orders (Table 6).

The interaction between cladode order and post-harvest storage period had a significant effect ($p < 0.05$) on the ash concentration in the forage cactus (Table 6). For the 'IPA-20' cultivar, the ash concentration ranged from 74 to 149 g kg^{-1} DM in second-order cladodes at 32 and 40 days of storage. In the case of the 'Miúda' cultivar, the ash concentration varied

Table 6. Cladode ash concentration (g kg^{-1} DM) of different forage cactus cultivars as affected by cladode order and post-harvest storage period

Post-harvest storage period (days)	'IPA-20'			'Miúda'		
	Cladode order					
	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
0	99aB	149aA	103aB	97bB	99aB	138aA
8	76aA	78cA	98aA	91bA	116bA	124aA
16	93aA	82cA	87aA	104abB	212aA	128aB
24	88aA	87cA	91aA	97bA	108bA	121aA
32	75aA	74cA	93aA	110abA	125bA	143aA
40	78aA	86cA	92aA	146aA	109bA	136aA
48	92aB	137bA	113aAB	108abB	127bAB	150aA
56	76aA	104bcA	97aA	104abA	121bA	132aA
<i>p value</i>						
Cultivars (C)	0.0002	0.0344	0.0943	0.0015	0.0001	0.0001
Period (SP)	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001
Order (O)	0.0027	0.0012	0.0001	0.0001	0.0001	0.0001
C × SP × O	0.0156	0.0341	0.0135	0.0073	0.0256	0.0073
Linear effect	0.2711	0.0149	0.1519	0.2687	0.3315	0.9278
Quadratic effect	0.4692	0.0124	0.1047	0.4191	0.2779	0.6945
Standard error	1.5392					

CV cultivar = 2.8%; CV cladode order = 3.4%; CV period = 5.8%.

*Means followed by the same letter (lowercase letters across columns and capital letters across rows) do not differ by Tukey test ($p > 0.05$).

from 91 to 212 g kg⁻¹ DM, recorded after 8 and 16 days of storage in first and second-order cladodes, respectively.

Ash concentration varied in cactus cladodes (Table 6). Pessoa et al. (2020) observed ash concentration of 82.8 to 95.5g kg⁻¹ DM for the cultivar 'IPA 20' in cladodes of different age. The mineral concentration of forage cactus can be influenced by the needs for physiological processes and the mineral content in the soil (Lédo et al., 2020).

Variations in chemical composition during storage periods can be too associated with the continuation of microbial respiration and aerobic activity. The possibility of cactus post-harvest storage represents an important technology to be applied by smallholders, with little variation in chemical composition of the stored material, with possible changes with increasing storage time and climatic and storage conditions.

Cladode anatomy

Interaction between post-harvest storage periods and cactus cultivar affected ($p < 0.05$) anatomical characteristics (Table 7). The epidermal area ranged from 10.3 to 62.8%, and from 8.8 to 51.2% for 'IPA-20' and 'Miúda', respectively. The parenchymal area ranged from 37.2 to 86.7%, and from 48.8 to 91.2% for 'IPA-20' and 'Miúda', respectively. 'IPA-20' and 'Miúda' presented different parenchymal areas for the majority of the tested post-harvest storage periods, with the exception of 24, 32, and 56 days. Epidermis and parenchyma area from 'Miúda' had quadratic effect regarding storage periods. Cultivar 'IPA-20' had no linear or quadratic effect (Table 7).

Epidermal and parenchymal areas varied during storage for both cultivars. This variation probably occurred as a result of air temperature, air relative humidity, and the experimental environment, as well intrinsic characteristics

of the evaluated cactacea. Cantwell (2001) reported that the storage of 'Nopalitos' 15 to 20 cm long (i.e., a cactus for human consumption as a vegetable) under natural conditions promoted yellowness and curving of the cactus cladodes, likely due to water loss. The 'IPA-20' and 'Miúda' cultivars had a similar proportion of epidermis and presented differences only during the post-harvest storage periods from 0 to 16 days and from 40 to 48 days. That indicated a possible interaction between cactus species and environmental conditions during storage. In general, after 32 days of storage, the proportion of epidermis on cactus cladodes tended to decrease presenting a quadratic response, while the portion of parenchyma increased linearly. This occurred probably due to the epidermis and cuticle are the outermost plant layers. Therefore, these layers are more affected by the surrounding environment, leading to modifications in their morphology as they adjust to the prevailing external conditions.

In cacti, epidermal cells have impermeable cutin located on the outer wall of the epidermis. It is a combination of lipidic substances with main function of plant protection against excessive water losses, as well as relief from solar radiation (Hills, 2001). By reducing the epidermal area, however, cladodes become more susceptible to bacterial entry, accelerating their deterioration during storage. This greater cladode deterioration was observed in the current experiment conform increasing storage periods (Table 1).

Silva et al. (2010) evaluated the anatomical characteristics of cactus cultivars with contrasting insect resistance and concluded that epidermal thickness was an important characteristic that contributes to insect resistance (*Dactylopius opuntiae* Cockerell). Although increasing epidermal thickness may increase cactus tolerance to insect attack, it may decrease cladode digestibility and reduce

Table 7. Epidermal and parenchymal area of forage cactus cladodes regarded cactus cultivars and post-harvest storage period

Post-harvest storage period (days)	Epidermis area (% of total area)		Parenchymal area (%)	
	'IPA-20'	'Miúda'	'IPA-20'	'Miúda'
0	30.1cB	43.5bA	69.8bA	56.5cB
8	62.8aA	51.1aB	37.2dB	48.8dA
16	30.6cB	51.2aA	69.3bA	48.8dB
24	42.5bA	50.2aA	57.5cA	49.8dA
32	28.3cA	29.2cA	71.7bA	70.8bA
40	13.3dB	26.9cA	86.7aA	73.0bB
48	13.4dA	8.7dB	86.8aB	91.2aA
56	10.3dA	9.9dA	89.6aA	90.0aA
<i>p</i> value				
Tissue (T)	0.0029	0.0001	0.0001	0.0049
Cultivars (C)	0.0001	0.0006	0.0001	0.0035
Period (P)	0.0001	0.0010	0.0001	0.0016
T × C × P	0.0006	0.0005	0.0029	0.0010
Linear effect	0.9523	0.2523	0.8994	0.3844
Quadratic effect	0.3310	0.0015	0.4125	0.0016
Standard error	0.4818		0.4059	

CV clone= 10.8%; CV period= 15.0%.

*Means followed by the same lowercase letters in the columns do not differ from each other using the Tukey test ($p > 0.05$) and means followed by the same uppercase letters from the rows, purchasing within each cultivar, do not differ from each other using the F test ($p > 0.05$).

feed intake by ruminants as a result. Perrotta & Arambarri (2018) observed for the *Opuntia* an epidermis smooth and uniseriate, covered by a thin cuticle and epicuticular waxes; a multiseriate hypodermis with one crystal layer; the cortex with external chlorenchyma, and internal hydrenchyma.

In addition to assessing differences in chemical and anatomical composition, it is recommended that future studies provide more comprehensive details about physiological functions during the storage process. Moreover, considering the pile size in the context of the deterioration process is important.

Conclusion

The cladodes of *Opuntia ficus-indica* Mill cv. 'IPA-20' and *Nopalea cochenillifera* Salm-Dyck. cv. 'Miúda' can be stored for up to 56 days without a reduction in crude protein and neutral detergent fiber concentrations. However, water-soluble carbohydrates (WSC) decreases conform increasing storage periods for both. The cladodes of 'Miúda', on the other hand, should not be stored for more than 30 days after harvesting due to greater rates of deterioration.

The deterioration of cladodes over the post-harvest storage period results in a reduction of WSC for both cultivars, which may compromise the nutritional quality of the stored material. This underscores the importance of proper management during the storage period to preserve the nutritional quality of forage cactus.

These findings highlight the variability in responses among different forage cactus cultivars to post-harvest storage technology for forage cactus, which represents an important practice to be applied by smallholders. The storage does not reduce cactus nutritive value to a great extent, possibly enabling a cost reduction in the processes of harvesting and transportation.

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Compliance with Ethical Standards

Author contributions: Conceptualization: MVFS, JCBDJ, MVC; Data curation: MVC; Formal analysis: MVC, DCS; Funding acquisition: MVFS; Investigation: MVFS, NGMS, MAL, JCLN; Methodology: MVFS, NGMS, MVC, DCS, MAL, JCLN; Project administration: MVFS, JCBDJ, MVC; Resources: MVFS, MAL; Supervision: MVFS, JCBDJ, MVC, DCS; Validation: MVFS, NGMS, JCBDJ, MVC; Visualization: MVFS, NVS; Writing

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