Journal of the Professional Association for Cactus Development

Size and harvest time of cladodes modulate the composition and physicochemical stability of prickly pear cactus mucilage

Lady Daiane Costa de Sousa[1], Kelem Silva Fonseca[1], Andréa Monteiro Santana Silva Brito[1], Yara Panta de Araújo[1], Jheizon Feitoza do Nascimento Souza[1], Lucas Vínicius Pierre de Andrada[1], Adriano do Nascimento Simões[1*]

[1]Academic Unit of Serra Talhada, Rural Federal University of Pernambuco, Gregório Ferraz Nogueira, José Tomé de Souza Ramos, PO Box: 063, 56909535, Serra Talhada, Pernambuco, Brazil.
*Corresponding author: Adriano.simoes@ufrpe.br

Abstract. The objective was to carry out a physicochemical characterization of the mucilage obtained from different sizes of cladodes harvested at different times. *Nopalea cochenillifera* Salm Dyck cladodes were collected in two sizes (100 to 230 and 240 to 300 mm), at two different times (6 am and 8 pm), and processed for mucilage extraction. This was dried in an oven, and then hydrated and kept at 5 °C for 12 days. The mucilage yield after harvest was quantified, in addition to the characterization of the main bands in the infrared region. In the mucilage, the following physicochemical analyzes were performed: K^+ and Na^+ content, electrical conductivity, titratable acidity, pH, total phenolic compounds, vitamin C, total soluble solids, total soluble carbohydrates, total soluble proteins after harvest (day 0), and to the 12 days of storage at 5 ºC. Correlation between the variables was determined through principal component analysis (PCA). The highest mucilage yield was obtained in cladodes harvested at 6 am, regardless of size. The 6 am harvest showed higher acidity and protein content and lower concentrations of soluble solids, EC, Na^+, K^+, vitamin C, carbohydrates, and phenolic compounds. In conservation, the mucilage of the cladodes with sizes between 100 and 230 mm and the harvest at 6 am showed greater stability. Principal component analysis (PCA) showed a tendency of formed groups between different sizes, harvest times, and conservation days, indicating that different conditions for obtaining the cladodes result in differences in the composition and stability of the mucilage. Thus, the results show that, in addition to the climatic conditions, as proposed by other authors, the harvest time and the size of the cladode modulate the physicochemical and important ion composition and stability, which can change the technological and industrial applications of mucilage.

Keywords: *Nopalea cochenillifera L.; Salm-Dyck; pH; Principal component analysis (PCA); Stability; Total soluble carbohydrates.*

Citation: Costa de Sousa, L. D., Silva-Fonseca, K., Silva-Brito, A. M. S, Panta-de Araújo, Y., do Nascimento-Souza, J. F., Pierre-de Andrada, L. V., do Nascimento- Simões, A. 2022. Size and harvestime of cladodes modulate the composition and physicochemical stability of prickly pear cactus mucilage. *Journal of the. Professional Association for Cactus Development. 24: 220-238*. https://doi.org/10.56890/jpacd.v24i.508.

Associate Editor: Alejandra Nieto-Garibay

Technical Editor: Tomas Rivas-Garcia

Received date: 03 June 2022

Accepted date: 28 July 2022

Published date: 30 August 2022

Introduction

The prickly pear cactus has various uses, as for example in Mexico, where it is used for human and animal food, with the production of young fruits and cladodes (Sáenz *et al.,* 2004). Differently in Brazil, a large part of the prickly pear cactus harvest is destined exclusively for animal feed (Nunes, 2011). In addition, prickly pear cactus has been applied in the pharmaceutical industry due to its potential as a wound healing agent (Ammar *et al.,* 2018; Di Lorenzo *et al.,* 2017), in effluent treatments acting as a flocculating agent in water contaminated with heavy metals (Nharingo and Moyo, 2016), in construction as an organic additive improving the properties of lime mortars(Ventol *et al.,* 2011), in medicine, in the treatment of diabetes, cancer, inflammatory and viral diseases (Feugang, 2006), in the fuel

industry as raw material for the production of bioethanol (Alencar *et al.,* 2018), in the packaging industry, biofilms and edible coatings (Gheribi *et al.,* 2018; Morais *et al.,* 2019), in the food industry, for food ingredients, with emphasis on its use of gluten-free biscuits (Dick *et al.,* 2020) and for bread formulation (Liguori *et al.,* 2020).

The mucilage of the prickly pear cactus is a natural source of polysaccharides that acts as a barrier to the transfer of water, decreasing the dehydration of food and maintaining its firmness. (Del-Valle *et al*., 2005). In addition, the use of mucilage as a raw material is renewable as long as its spread continues and environmentally friendly (Gheribi and Khwaldia, 2019), presenting characteristics such as viscosity, elasticity, emulsifying properties, and water holding capacity (Medina-Torres et al., 2000; Sáenz *et al.*, 2004). It is extremely important to know the physicochemical properties of mucilage in terms of electrical conductivity, pH, sodium and potassium content, and how these variables interfere with the molecular conformation of mucilage. In addition, the characterization of proteins and carbohydrates is necessary since the polysaccharides provide a barrier to O2 and the mechanical characteristics essential to the constitution of a good film (Bertan *et al.,* 2005).

Recent research has shown the physicochemical change of mucilage of the *Opuntia* genus according to climatic conditions (Du Toit *et al.*, 2018; Du Toit *et al.*, 2020). The months of harvest and the cultivar are imperative to change the physicochemical and technological properties of the mucilage of the genus *Opuntia* (Du Toit *et al.*, 2019). In addition, Sandorval *et al.* (2019) showed that genotypes of the genus *Opuntia* produce edible films with different physicochemical characteristics. However, the studies cited above were with the cactus of the genus *Opuntia* in South Africa and Mexico. Recently, in Brazil, studies with the genus *Nopalea* showed that the time of year (Panta-Araújo *et al*., 2021) and the size of the cladode (data not yet published) influence the physicochemical composition of the mucilage. In addition, cactus have a carbon metabolism called Crassulacean Acid Metabolism (CAM), in which the composition of organic acids changes in a few hours (Rodriguez-Felix and Cantwell, 1988), the time of harvest can also change the physicochemical composition of the mucilage. Thus, it is important to prove that other managements little mentioned in the works with cladodes of the genus *Nopalea* are important for the physicochemical changes of mucilage and consequently can change the technological and industrial properties, as suggested by Du Toit *et al*. (2019). Therefore, it is believed that management associated with harvest time and cladode size, results in mucilage yields and altered quality. These factors are imperative to define the technological application of mucilage in the industry. In addition, storage of mucilage under refrigeration can be an important technique to keep the mucilage chemically stable for application as edible films.

The objective of this study, then, was to evaluate the physiochemical properties of mucilage extracted from the Miúda clone, *Nopalea cochenillifera* (L.) Salm-Dyck, using different sizes of cladodes harvested at different times and kept under refrigeration.

Material and Methods

Raw material and weather conditions

Cladodes of prickly pear cactus, clone Miúda, *Nopalea cochenillifera* (L.) Salm-Dyck, were collected from the experimental area of the Federal Rural University of Pernambuco / Academic Unit of Serra Talhada (UFRPE / UAST) in Serra Talhada, Pernambuco, Brazil. According to the Köppen classification, the region's climate is of the BSh type, characterized as semi-arid, hot, and dry, with

https://www.jpacd.org Electronic ISSN: 1938-6648

average annual temperatures over 25 ºC, an average annual precipitation of 647 mm, and with an altitude of 481 m (Beck *et al.,* 2018). The climatic conditions, average temperature, relative humidity, and rainfall during the experimental period are described in Figure 1.

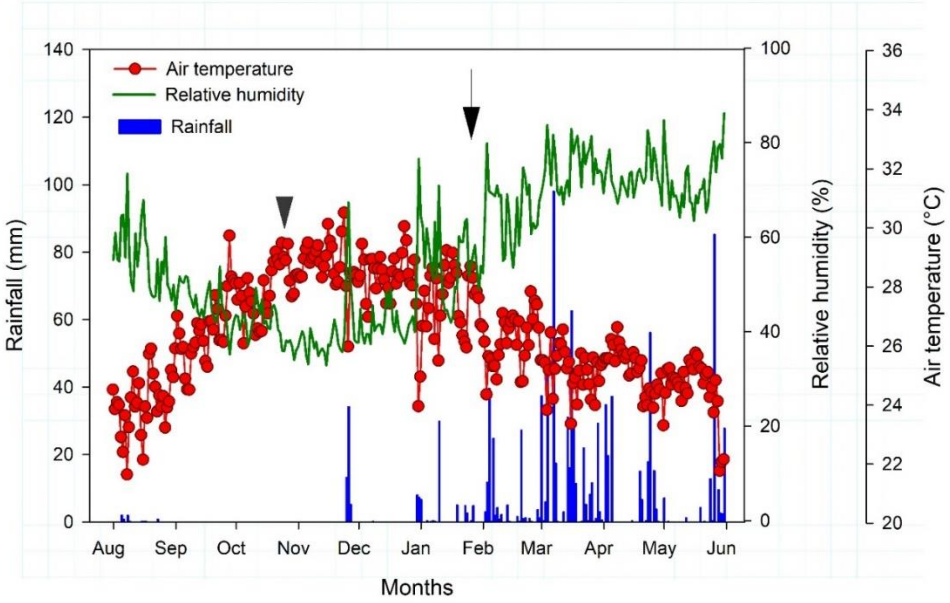

Figure 1. Air temperature (°C), relative humidity (%), and rainfall (mm) between the months of August 2019 to June 2020 in the municipality of Serra Talhada, Pernambuco, Brazil. Source: Instituto Nacional de Meteorologia (INMET), 2020. The arrows indicate the months in which the harvests were carried out.

Physicochemical evaluation of mucilage from Prickly pear cactus

It was necessary to divide this work into specific studies on the characterization of mucilage: the experiment related to the size of the cladodes was carried out first. The cladodes were collected in the month of November 2019 and selected by size in terms of the variables chosen to be studied. Once the size was defined, the second study was carried out related to the harvest time, February 2020.

Harvesting two sizes of cladodes and storage

The cladodes of prickly pear cactus with sizes between 100 to 230 mm in length and between 240 to 300 mm in length were harvested at 6 am. After harvesting, they were processed according to the methodology defined by Panta-Araújo *et al.* (2021), with modifications. The cladodes were weighed and the epidermis was removed. Then, the parenchyma was weighed, cut into cubes, and homogenized in a multiprocessor (Philips Walita, ri7775, Barueri, Brazil), for 30 s, at a proportion of 2:3 (two parts of mucilage for three of 84% ethyl alcohol, reused - used in previous extractions). The homogenate was filtered and washed in reused ethyl alcohol. The precipitate was collected and dried in an oven at 55 ºC for 24 h. Afterward, the dry powder was pulverized using a portable mill (Polespresso, Original coffee flavor, Carapin da Serra, Brazil).

The powdered mucilage was hydrated in distilled water (4% w/v) and kept in Petri dishes covered with plastic film, with 25 mL of mucilage in each dish. The mucilage was kept refrigerated at 5 ºC and the analyses were made on day 0 and after 12 days.

Harvesting of cladodes at two times and Storage

In the second study, cladodes with sizes between 100 and 230 mm in length (as in the previous study) were used. The cladodes were collected at two different hours, 6 am and 8 pm, and the mucilage was extracted as already described. Subsequently, the powdered mucilage was hydrated in distilled water (4% w / v) and kept in Petri dishes covered with plastic film, with 25 mL of mucilage on each plate. The mucilage was kept under refrigeration at 5 °C and the analyzes were performed on day 0 and after 12 days.

Agroindustrial yield

The mucilage yield was determined based on fresh weight, using as a base the entire cladode and pieces of the parenchyma, using the following equations:

$$CY = \frac{MPM}{FCM} 100 \qquad (1)$$

Where FCY = Fresh Cladode Yield, %; MPM = Mass of Powder Mucilage, g; FCM = Fresh Cladode Mass, g. YSP =MPM⁄MCP*100 Where YCP = Yield of Cladode Parenchyma, %; MPM = Mass of Powder Mucilage, g; MCP = Mass of the Cladode Parenchyma, g.

Total soluble solids (TSS), Titratable acidity (TA), pH, and Vitamin C (vit. C)

The content of total soluble solids in the hydrated mucilage was obtained through the use of a portable digital bench refractometer (INSTRUTHERM, RTD-95, São Paulo, Brazil) in which approximately 1 mL of mucilage was used to perform the reading. The results were expressed as a percentage of soluble solids.

Titratable acidity was performed according to Astello-García et al. (2015), with some modifications using 0.1 N (NaOH) aqueous hydroxide solution. The results were calculated by the following formula and expressed in % citric acid.

$$A = \frac{NVEq\ citric\ acid}{v} \qquad (2)$$

Where TA = titratable acidity, % citric acid; N = NaOH concentration; V = Volume of NaOH used in the titration, mL; Eq = equivalent grams of citric acid, 64.02; v = Volume of the sample used mL.
The pH was measured using a pH meter (TECNAL, TEC-5, Piracicaba, Brazil). The sensor was placed inside the Petri dishes containing the samples.

The vitamin C content was determined by titration, using Tillman's solution according to the method described by (Lutz, 2008). Was expressed in milligrams of ascorbic acid per 100 g of dry mass (mg $100g^{-1}$) and calculated using the formula below: AA=VxFx100⁄A. Where AA = mg ascorbic acid $100g^{-1}$ MS; V = Volume of Tillman's solution spent on titration, mL; A = Volume of the sample used, mL; F = Tillman's solution factor. In which the factor of Tillman's solution was calculated by the formula below:

$$F = \frac{VitC}{ST} \qquad (3)$$

https://www.jpacd.org Electronic ISSN: 1938-6648

Where F = factor of the Tillman's solution; Vit C = amount of vitamin C solution used in the titration, mg; ST = spent Tillman's solution, mL.

Electrical conductivity (EC), Sodium (Na^+) and Potassium (K^+)

Electrical conductivity was performed using a bench conductivity meter (TECNAL, Tec-4MP, Piracicaba, Brazil). The results were expressed in mS cm^{-1}.

The sodium and potassium levels were obtained according to the methodology described by (Lutz, 2008), which is based on the flame emission photometry technique using a flame photometer (MICRONAL, B462, Piracicaba, Brazil). Approximately an aliquot of 5000 µL was used, this volume being composed of 4900 µL of pure water and 100 µL of mucilage. The results were expressed in mg of K^+ or Na^+ 100 g dry mass and quantified based on the equation obtained for the standard curve.

Total soluble carbohydrates (TSC), Total soluble proteins (TSP), and Total phenolic compounds (TPC)

The determination of total soluble carbohydrates followed the methodology proposed by Dubois et al. (1956), with some modifications. Two milliliters of hydrated mucilage were centrifuged (Hettich, MIKRO 220, Berlin, Germany) at 10,000 rpm, at 4 ° C for 21 min. A 10 µL aliquot was added to 490 µL of deionized water, 500 µL of phenol (5%), and 2500 µL of sulfuric acid P.A. The tubes were vortexed (TECNAL, AP56, Araraquara, Brazil) and kept at rest for 10 min. The readings were performed on a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 490 nm and the total carbohydrate content was expressed in g of soluble carbohydrates 100g of dry mass.

The content of total soluble proteins was obtained through the methodology described by Bradford, (1976), with some adaptations. A 2 mL volume of hydrated mucilage was centrifuged (Hettich, MIKRO 220, Berlin, Germany) at 10,000 rpm at 4 ºC for 21 min.

One hundred microliters of the supernatant were mixed with 1000 µL of Bradford reagent. The tubes were vortexed (TECNAL, AP56, Araraquara, Brazil) and kept at room temperature for 15 min. The readings were performed using a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 595 nm. Bovine serum albumin (BSA) was used as an external standard. The total soluble protein content was expressed in mg of soluble protein 100g dry matter.

The determination of the content of total phenolic compounds was carried out according to Jaramillo-Flores *et al.* (2003), with some modifications. A 2 mL volume of hydrated mucilage was centrifuged in a centrifuge (Hettich, MIKRO 220, Berlin, Germany) at 10,000 rpm, at 4 ° C for 21 min. A 150 µL aliquot of the supernatant was combined with 2400 µL of deionized water and 150 µL of Folin Ciocalteu reagent (0.25 M). The mixture was homogenized in a vortex (TECNAL, AP56, Araraquara, Brazil) for 3 min and 300 µL of sodium carbonate (1 M) was added. The tubes were kept in the dark at room temperature for 2 h. The readings were performed on a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 725 nm. The content of total phenolic compounds was expressed in mg of gallic acid 100 g dry matter.

Fourier transform infrared spectroscopy (FTIR)

Spectral analyzes in the mid-infrared region were performed with a Fourier transform infrared (FT-IR) spectrophotometer (Perkin Elmer® Frontier), using the universal attenuated total reflection (UATR) accessory. The spectra were obtained in the region of 4000-400cm^{-1}, resolution 8 cm^{-1}, and with 8 scans. FTIR analysis was performed on samples of the mucilage powder only on day 0. The blank

https://www.jpacd.org Electronic ISSN: 1938-6648

was obtained using air and measurements were made in quadruplicate, directly on the white mucilage powder under the diamond crystal.

Experimental design and statistical analysis

The experiments were arranged in each study in a completely randomized design, in a 2x2 factorial scheme, with four replications. In the first study there were two sizes of cladodes (100 to 230 mm in length and 240 to 300 mm in length) and in two days of storage (days 0 and 12). In the second study, there were two harvest times (6 am and 8 pm) and two conservation days (0 and 12 days). Data were submitted to Shapiro-Wilk normality tests. Subsequently, the data were submitted to analysis of variance (ANOVA) at a significance level of 5%, using the Fisher-Snedecor F test. When significant, the means were submitted to the Tukey test at 5% probability. Principal component analysis (PCA) was obtained through physical-chemical integration and subsequent decomposition into orthogonal vectors. The results of the correlation matrix were presented in biplots with their distribution in the order space, variances, and Pearson's correlation. For statistical analysis, the R version 4.1.3 software was used. Plots were created using Sigma Plot software, version 14.

Results

Statistical analyzes (Table 1) showed effects of interaction and isolated factors related to treatments: sizes of cladodes, harvest time, and conservation time on the physicochemical aspects analyzed in the mucilage of prickly pear cactus.

Table 1. Summary of analysis of variance (ANOVA) for the effects of cladode sizes (100 to 230 mm and 240 to 300 mm), harvest times (6 am and 8 pm), storage time (0 and 12 days), and their interactions with respect to potassium content (K^+), sodium content (Na^+), electrical conductivity (EC), titratable acidity (TA), content of total phenolic compounds (TPC), total soluble solids (TSS), total soluble carbohydrates (TSC), pH, vitamin C content and total soluble protein (TSP) content of the mucilage of prickly pear cactus (clone Miúda).

STUDY 1 - SIZE OF CLADODIOS										
FACTORS	K^+	Na^+	EC	TA	TPC	TSS	TSC	pH	VIT.C	TSP
Cladode Size	NS	NS	**	*	NS	**	**	NS	**	**
Storage time	**	NS	**	*	NS	*	**	NS	**	**
Size x Storage	NS	NS	NS	NS	NS	*	**	*	*	*
STUDY 2 - HARVEST TIME										
FACTORS	K^+	Na^+	EC	TA	TPC	TSS	TSC	pH	VIT.C	TSP
Harvest Time	**	**	**	**	**	**	**	**	*	NS
Storage time	NS	**	**	**	NS	NS	NS	**	**	**
Time x Storage	*	**	**	**	*	NS	NS	**	**	**

NS - not significant, * p <0.05, ** p <0.01

https://www.jpacd.org Electronic ISSN: 1938-6648

Effect of the size of cladodes on the physicochemical properties of mucilage

There was no significant difference in agroindustrial yield between the two sizes studied (Table 2). There was no interaction effect among the factors studied for the K^+, Na^+, EC, citric acid, and total phenolic compounds present in the mucilage (Table 1). Because of this, Figures 2 and 3 show only the effect of size and storage days. The mucilage extracted from cladodes between 100 and 230 mm in size showed significantly less electrical conductivity compared to cladodes with sizes between 240 and 300 mm (Figure 2E). During storage, the K^+ content increased and the EC decreased significantly (Figures 2B and F).

Table 2. Agroindustrial yield (%) of the mucilage of prickly pear cactus (clone Miúda) in two sizes of cladodes (100 to 230mm and 240 to 300mm) and two harvest times (at 6 am and 8 pm).

Cladode size (mm)	Agroindustrial yield (%)	
	Fresh Cladode	Cladode Parenchyma
100-230	3.99 ±1.2 a	9.80±1.7 a
240-300	4.13±1.0 a	10.79±2.7 a
Harvest Time	Fresh Cladode	Cladode Parenchyma
6 am	3.99 ±1.2 a	9.80±1.7 a
8 pm	2.36 ±1.0 b	4.35±1.2 b

Means followed by the same lower case letter in the column do not differ from each other by the Tukey test, at the level of 5% probability, ± represents standard deviation of the means.

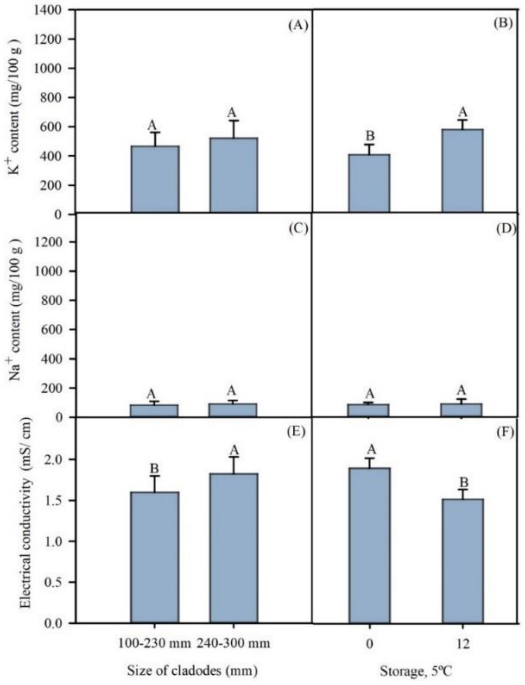

Figure 2. Potassium content, K^+, sodium content, Na^+ and electrical conductivity, EC of mucilage extracted from cladodes of the clone Miúda of different sizes (A, C, and E) and on different days of storage (B, D, and F). The bars represent the standard error of the mean. The different letters indicate a significant difference by the Tukey test ($p < 0.05$).

https://www.jpacd.org Electronic ISSN: 1938-6648

The citric acid content was higher for cladodes between 240 and 300 mm in size (Figure 3A). On the other hand, the content of total phenolic compounds did not differ between the smaller and the larger sizes (Figure 3C). In addition, under storage conditions, there was an increase in the citric acid content (Figure 3B).

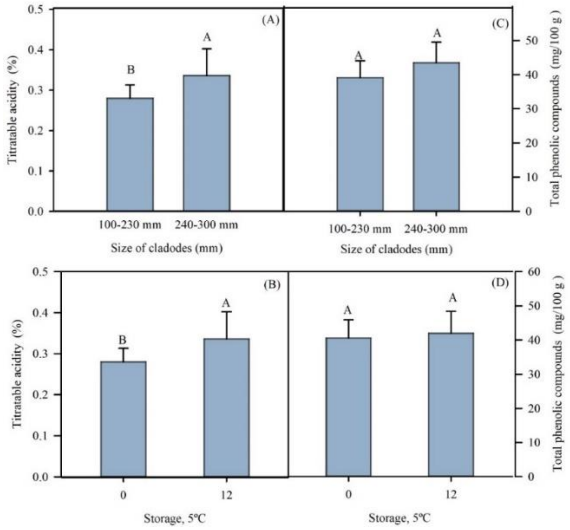

Figure 3. Titratable acidity (%) and total mucilage phenolic compounds extracted from cladodes of the Miúda clone of different sizes (A and C) and on different storage days (B and D). The bars represent the standard error of the mean. The different letters indicate a significant difference by the Tukey test ($p < 0.05$).

There was an interaction effect between the factors studied for the levels of total soluble solids, total soluble carbohydrates, pH, vitamin C and total soluble proteins (Table 1). The amounts of total soluble solids and total soluble proteins were significantly higher for sizes from 100 to 230 mm, compared to sizes from 240 to 300 mm, at the beginning of the experiment (Figures 4A and E). The smaller cladodes, on the other hand, had the lowest levels of vitamin C shortly after harvest (Figure 4D). The pH values and soluble carbohydrates did not differ in relation to size (Figures 4B and C). After 12 days of mucilage storage, the levels of total soluble solids, total soluble carbohydrates and total soluble proteins, remained stable for mucilage of the cladodes from 100 to 230 mm (Figures 4A, B, and E). The pH increased in the mucilage obtained from cladodes from 100 to 230 mm (Figure 4C). On the other hand, the vitamin C content decreased during storage for both sizes (Figure 4D).

https://www.jpacd.org Electronic ISSN: 1938-6648

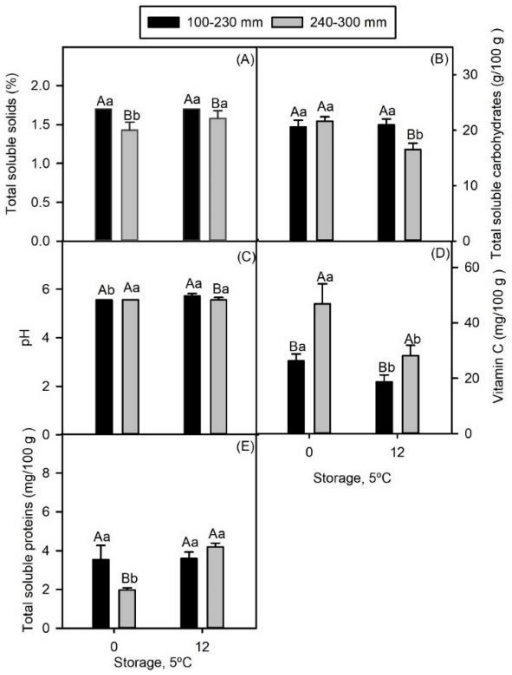

Figure 4. Total soluble solids (A), total soluble carbohydrates (B), pH (C), vitamin C content (D), and total soluble proteins (E) of the mucilage extracted from cladodes of the clone Miúda of two different sizes and harvested at 6 am. The bars represent the standard error of the mean. Capital letters compare sizes and lower-case letters compare storage days.

The main bands in the FTIR spectrum of mucilage obtained from cladodes between 100 and 230 mm and 240 to 300 mm in size, at both collection times (6 am e 8 pm), were the following: 3334 cm^{-1}; 2926 cm^{-1}; a more intense one at 1612 cm^{-1} and another weaker at 1426 cm^{-1} (Figure 5). In addition, a set of peaks in the region between 1380 and 1240 cm^{-1} and 1044 cm^{-1} can be seen (Figure 5).

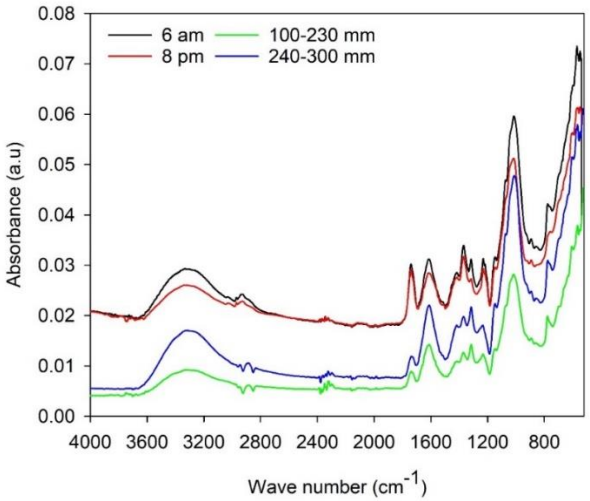

Figure 5. FTIR spectra of mucilage (A) and Scores graph obtained by the PCA of 16 spectra of powder mucilage (B) extracted from clone Miúda obtained from cladodes between 100 and 230 mm and 240 to 300 mm in size, harvested at different times (6 am and 8 pm).

https://www.jpacd.org Electronic ISSN: 1938-6648

Influence of harvest time on the physicochemical properties of mucilage

In the harvest time study, there was no interaction between harvest time and storage days for the content of total soluble solids and total soluble carbohydrates (Table 1). On the other hand, the other analyses showed significant interactions (Table 1).

It was observed that the agroindustrial yield, both based on the whole cladode, and based on the parenchyma, was higher for the mucilage obtained from the cladodes harvested at 6 am (Table 2). Harvest at 8 pm increased the levels of soluble solids and total soluble carbohydrates in the mucilage (Figure 6A and C) and these parameters remained stable during storage (Figure 6B and D).

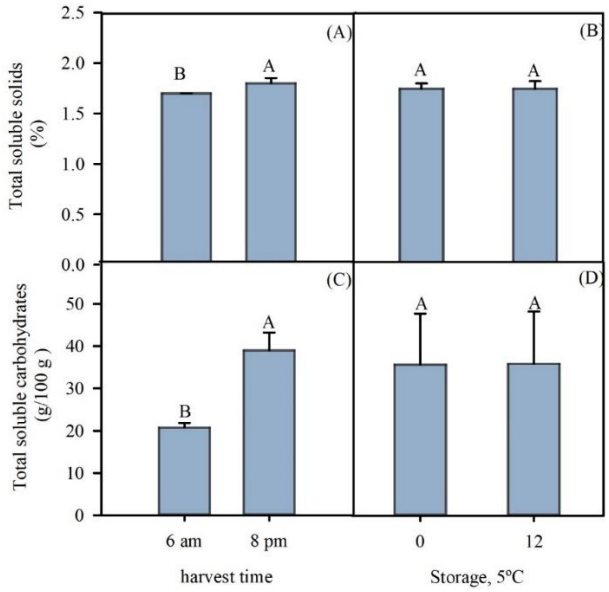

Figure 6. Content of total soluble solids and total soluble carbohydrates of mucilage extracted from cladodes between the clone Miúda, 100 and 230 mm in size, collected at different times (A and C) and on different days of storage (B and D). The bars represent the standard error of the mean. The different letters indicate a significant difference by the Tukey test ($p < 0.05$).

At harvest time at 6 am, the EC and K^+ contents in the mucilage were lower, in relation to the harvest at 8 pm (Figures 7 A and B). On the other hand, there was no difference between the two harvest times in terms of pH and sodium content (Figure 7 C and D). In storage, only the cladodes harvested at 6 am remained with a stable pH and Na^+, compared to those collected at 8 pm (Figure 7 C and D). For cladodes harvested at 6 am, EC decreased during storage, while the K^+ content increased, (Figure 7 A and B).

The harvest at 8 pm, at the beginning of the experiment, increased the levels of vitamin C and total phenolic compounds in the mucilage (Figure 7 E and F). On the other hand, harvesting the cladodes at 6 am resulted in higher titrable acidity and protein content (Figure 7 G and H). Under storage, only the cladodes harvested at 6 am retained stability of the total phenolic compounds and total soluble proteins, compared to those collected at 8 pm (Figure 7 F and H). The vitamin C content decreased under storage for mucilage harvested at both times (Figure 7 E). The titrable acidity decreased under storage only for the mucilage obtained from the harvest at 6 am (Figure 7 G).

https://www.jpacd.org Electronic ISSN: 1938-6648

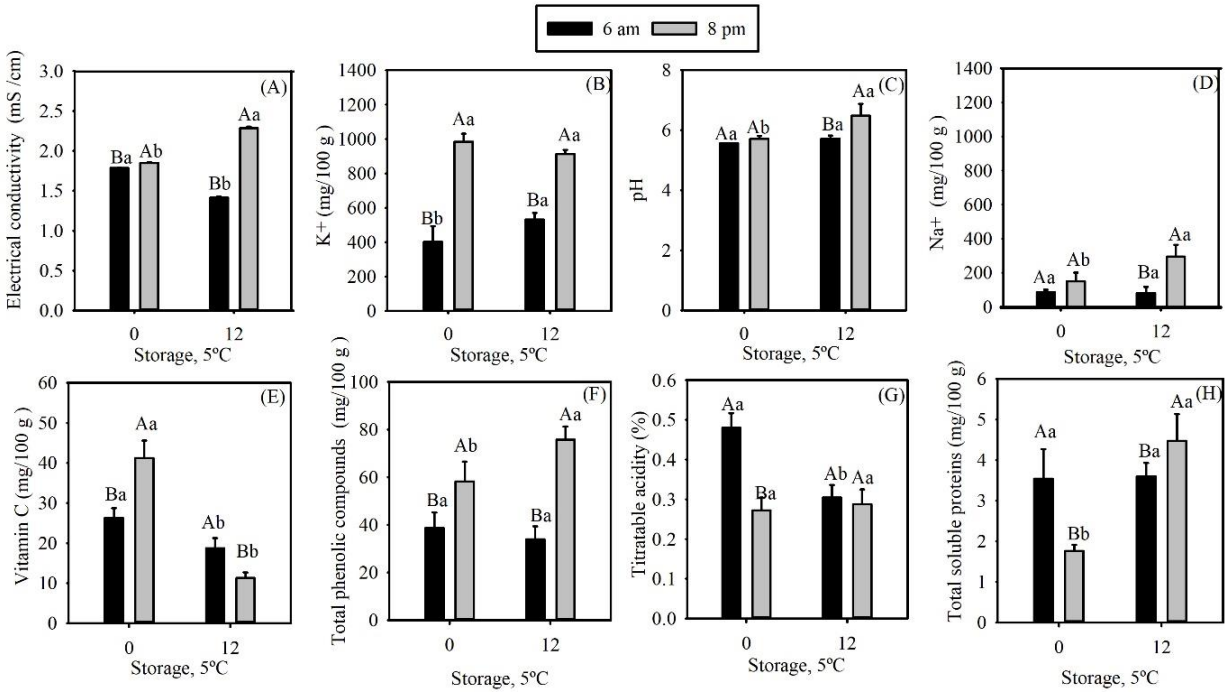

Figure 7. Electrical conductivity (A), K^+ (B), pH (C), Na^+ (D), vitamin C content (E), total phenolic compounds (F), titrable acidity (G), total soluble proteins (H) mucilage extracted from cladodes of the clone Miúda between 100 and 230 mm in size, harvested at 6 am and 8 pm and on different days of storage. The bars represent the standard error of the mean. Capital letters compare harvest time and lower-case letters compare storage days.

In the PCA that included cladode sizes (100 to 230 and 240 to 300 mm) and storage days (0 and 12 days), 89.81% of the total variation in the data was explained by two main components (PC1=52, 78%, and PC2=37.03%) (Figure 8A). The cladodes with sizes from 100 to 230 mm evaluated on storage days 0 and 12 were positively correlated with each other and showed a positive correlation with SS and pH and a negative correlation with AT, Vit C, TPC, and Na^+ (Figure 8A). CT showed a positive association with cladodes with a size of 240 to 300 mm assessed on day 0 (Figure 8A). In turn, they correlated negatively with the variables K^+ and TSP, which were positively correlated with each other and with the cladodes of size 240 to 300 mm evaluated on day 12 (Figure 8A). In the PCA that included harvest times (6 am and 8 pm) and conservation days (0 and 12 days), 91.61% of the total variation in the data was explained by two main components (PC1=63.83% and PC2=27.76%) (Figure 8B). The 6 pm collection times evaluated on storage days 0 and 12 were positively correlated with each other and with AT, and negatively correlated with K^+, TC, and SS (Figure 8B). These variables, in turn, were positively correlated with each other and with the 8 pm harvest time evaluated on day 0 (Figure 8B). There was a positive correlation between the variables TSP, Na^+, pH, EC, and TPC and the time of collection at 8 pm. However, these parameters were negatively related to the Vit C content (Figure 8B).

https://www.jpacd.org Electronic ISSN: 1938-6648

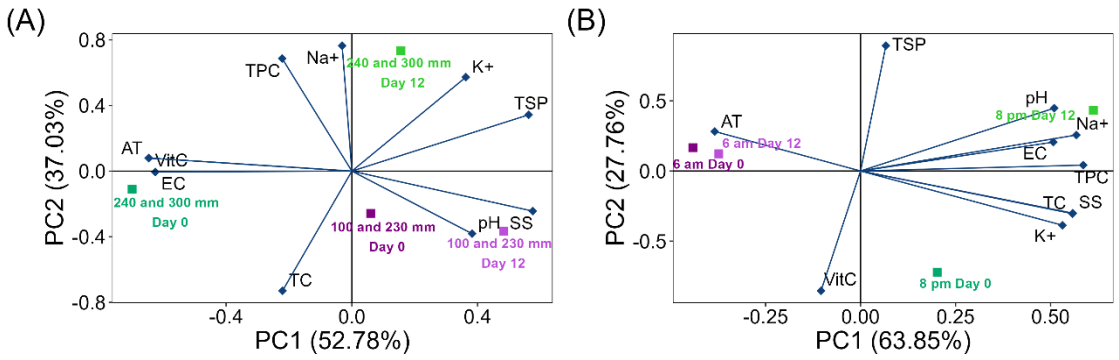

Figure 8. Scores obtained by PCA from physicochemical data on the mucilage of cladodes of the Miúda clone, collected in different sizes (100 and 230 mm and 240 to 300 mm) and on different follow-up days (0 and 12 days) (A). PCA scores of physical-chemical mucilage data from the Miúda clone, collected at different times (6 am and 8 pm) and on different breastfeeding days (and 12 days) (B).

Discussion

The present work focused on the sizes and times of cactus harvest as factors that modulate the mucilage yield, physicochemical properties, and stability of hydrated mucilage and stored for 12 days at 5°C. A major challenge in obtaining mucilage from prickly pear cactus relates to mucilage yield. A smaller yield may not be economically attractive for use as an alternative method to obtain polymers from renewable natural sources. In the present study, the highest yield was around 10%, using fresh parenchyma as a reference, regardless of size, from cladodes harvested at 6 am (Table 2). This value was more than double in relation to the cladodes harvested at 8 pm (Table 2), and almost seven times higher, in relation to that obtained by Sepúlveda *et al.* (2007) and Dick *et al.* (2020). In the literature, the highest yields observed are the studies by Petera *et al.* (2015) who obtained a 24% yield based on the powder from dry cladodes. Regarding the fresh matter, the highest yields were obtained by Sepúlveda *et al.* (2007) and Dick *et al.* (2020) with values of 1.56% and 1.20%, respectively.

It is known that cacti are drought tolerant, this is due in part to the storage of water and nutrients in the parenchyma during the rainy season (Ribeiro *et al.,* 2010) and the emission of adventitious roots (Snyman, 2006). In the present study, the mucilage yield did not depend on the size of the cladode. In addition, smaller cladodes have less fiber and lignin content, being more tender and soft, which facilitates the mucilage extraction process (Silva *et al.,* 2015) enabling easier handling during the processing of cladodes. For this reason, cladodes with sizes between 100 and 230 mm were selected, for the continuation of the experiments. The morning harvest had a higher yield in mucilage (Table 2), showing that, for mucilage extraction, an early morning harvest is more promising (Silva *et al.,* 2015). This may be associated with higher levels of organic acids and proteins in the cladodes (Figure 7 G and H), since in these plants, a stomatal opening occurs during the night in order to accumulate organic acids (Lüttge, 2010), resulting in water loss through transpiration, and consequently, the cladodes harvested at 6 am have low turgidity, increasing the yield due to the concentration of the compounds (Scalisi *et al.,* 2016). This can be reflected in the mucilage yield values observed throughout the day in the present work.

In the present work, the two sizes studied had similar carbohydrate content (Figure 4B). However, the smaller sized cladodes, between 100 and 230 mm, showed higher levels of soluble solids and proteins (Figures 4A and E). The protein content of cladodes tends to decrease with age, which may be related

to greater metabolic activity in the early stages of maturation (Acevedo *et al.,* 1983), and to the transport of nitrogen from mature tissues to the young plants (Acevedo *et al.,* 1983; Figueroa-Pérez *et al.,* 2018). This may explain the higher protein content in cladodes of smaller sizes, between 100 and 230 mm (Figure 4E).

Phenolic compounds are relevant phytochemicals, as they play an important role in antioxidant capacity (Nabil *et al.,* 2020). High concentrations of phenolic compounds, ranging between 118 and 126 mg GAE 100 g^{-1}, favor the incorporation of mucilage in foods such as cookies and bread (Dick et al., 2020; Liguori *et al.,* 2020). On the other hand, for use in the formulation of edible films, the increase in phenolic compounds may not be interesting because these compounds can react with the polysaccharides present in the mucilage, decreasing the formation of films and increasing the water barrier properties (Jaramillo-Flores *et al.,* 2003; Nabil *et al.,* 2020). However, the elimination of phenolic compounds for the production of edible films is a great challenge, given that the fraction of polysaccharides in mucilage is strongly associated with phenolic components through ester bonds with galactose and arabinose residues (Jaramillo-Flores *et al.,* 2003; Manhivi *et al.,* 2018; Nabil *et al.,* 2020). Changes in this polysaccharide association can influence the multiple functionalities of mucilage (Manhivi *et al.,* 2018). The night harvest of the smaller sizes of cladodes significantly increased the content of soluble solids, soluble carbohydrates, and total phenolic compounds (Figures 6A e C and 7F). Thus, based on the phenolic compounds, if the mucilage is intended for film production, both sizes can be harvested. On the other hand, if the mucilage is to be incorporated into foods, with the aim of increasing functional properties, night harvesting would be the best option.

Electrical conductivity estimates the concentration of ions present in the mucilage. Variations in this parameter can be attributed to the presence of divalent and monovalent ions (Monrroy *et al.,* 2017). This parameter showed that the size from 100 to 230 mm showed lower EC (Figure 2E). This can be explained by the physiological stage: the younger cladodes present a greater flow of water, causing dilution in ions concentration (Scalisi *et al.,* 2016). In addition, the harvest performed at 6 am also presented lower mean EC values (Figure 7A). In the present work, the K^+ and Na^+ ions were measured, which are ions that contribute to the observed EC values, including others, such as Ca^{2+} and Mg^{2+} (Monrroy *et al.,* 2017). The Na^+ content did not change regardless of the cladode size (Figure 2C) and of the harvest time (Figure 7D). However, the K^+ content did not differ by size (Figure 2A), but by time, showing high values in the mucilage of cladodes harvested at 8 pm (Figure 7B). This may be associated with the stomatal opening induction process, which occurs in cacti during the night (Males and Griffiths, 2017). In mucilage, the presence of electrolytes is important for the formulation of suspensions (Monrroy *et al.,* 2017), since the electrical conductivity directly influences viscosity. The viscosity depends directly on the ionic strength because the concentration of ions or salts present in a solution causes a breakdown in the molecular conformation (Van Krevelen, 1997) which is not ideal for the use of this mucilage in the formulation of films and coatings due to the decreased adhesion capacity coating the product surface (Assis and Britto, 2014). Thus, on the basis of the electrical conductivity of the mucilage. The production of films and coatings would be more suitable from cladodes harvested smaller (100 to 200mm) harvested in the morning (6 am).

The FTIR spectra provide a profile of the main functional groups present in the mucilage (Rodríguez-González *et al.,* 2014 and Gheribi *et al.,* 2018). In these studies, the specific frequencies of the functional groups present in the mucilage were verified, such as carboxylic acid, carboxylate, ether groups, and alcohol. For both sizes and times studied, a small peak was found in the region of 1734

cm^{-1} (Figure 5), characteristic of mucilage with a certain degree of esterification which is not indicated for the production of edible mucilage films, since a higher degree of esterification, the carboxyl groups will not be free to interact with water molecules, causing a low absorption capacity (Gheribi *et al.,* 2018). The cladodes of smaller size showed a lower peak in the region 1734 cm^{-1} (Figure 5), indicating a lower degree of esterification. Furthermore, in the mucilage, the presence of carboxylate ion (COO^-) was found, giving rise to two bands, one more intense at 1612 cm^{-1}, arising comes from asymmetric axial deformation, and another weaker band at 1426 cm^{-1}, which comes from symmetric axial deformation (Rodríguez-González *et al.,* 2014). In addition to a set of peaks in the region between 1380 and 1240 cm^{-1} corresponding to vibrations C-H and O-H, there is also a high intensity at 1044cm^{-1}, which corresponds to the vibrations of C-C and C-O present in cactus mucilage powder (Figure 5). There are also untied bands: at 3334cm^{-1} attributed to the OH stretch of alcohol and carboxylic acid; 2926cm^{-1} attributed to the vibrations of C-H bonds, which includes symmetrical and asymmetric stretching of C-H, CH_2, and CH_3 bonds of molecules present in the mucilage (Gheribi *et al.,* 2019; Rodríguez-González *et al.*, 2014).

The present study demonstrated how cladode size and harvest time influence physicochemical characteristics and mucilage stability. In the analysis of the principal components (PCA), it is possible to notice a tendency of groups formed between the different sizes, harvest hours, and conservation days (Figure 8), reinforcing the changes in the physicochemical composition of the mucilage in response to the adopted agronomic management practices. The grouping of the physicochemical variables of cladodes with sizes from 100 to 230 mm (Figure 8A) and harvested at 8 pm (Figure 8B) on the evaluated storage days reinforces the greater stability of the mucilage obtained from this size. The mucilage obtained from cladodes at 6 am resulted in lower values for electrical conductivity (Figure 7A) and phenolic compounds content (Figure 7F), mainly in the smallest cladodes (100 to 200mm) (Figure 2E). This may be indicative of a more suitable mucilage for the production of films and coatings. However, studies are needed to prove this indication. On the other hand, the mucilage obtained from the harvest at 8 pm resulted in higher levels of total soluble solids (Figure 6A), total soluble carbohydrates (Figure 6C), total phenolic compounds (Figure 7F), vitamin C (Figure 7E), electrical conductivity (Figure 7A) and potassium content (Figure 7B). This may suggest that the night harvest may be more suitable for the application of mucilage for incorporation into foods, to improve the antioxidant potential, due to its higher content of phenolic compounds. However, more studies are needed.

Conclusions

The highest mucilage yield was obtained in cladodes harvested at 6 am, regardless of size. This time also provided mucilage with higher acidity and protein content and lower concentrations of soluble solids, EC, Na^+, K^+, vitamin C, carbohydrates, and phenolic compounds. In conservation, the mucilage of the cladodes with sizes between 100 and 230 mm and the harvest at 6 am showed greater stability. Principal component analysis (PCA) showed a tendency of formed groups between different sizes, harvest times, and conservation days. Thus, the results show that, in addition to the climatic conditions, as proposed by other authors, the harvest time and the size of the cladode modulate the physicochemical and important ion composition and stability, which can change the technological and industrial applications of mucilage.

https://www.jpacd.org Electronic ISSN: 1938-6648

Acknowledgments

To the Grupo de Instrumentação e Análise Química (UAST / UFRPE) and the teacher Maria Fernanda Pimentel Avelar (DEQ/UFPE) pelo uso dos softwares The Unscrambler X, versão 10.2 (CAMO S.A.) e MATLAB R2010a 7.10.0.499 (MathWorks, Inc., USA).

Ethics statement

Not applicable

Consent for publication

Not applicable

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

FUNDING

This research was supported by the CAPES- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- (Proc. 88881-159183/2017-01); FACEPE- Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco- (PQ-0795-5.01/16); UFRPE- Universidade Federal Rural de Pernambuco- (PRPPG 015/2018) and CNPq- Conselho Nacional de Desenvolvimento Científico e Tecnológico- (423100/2018-1); FACEPE (PRONEX/NUQAAPE (APQ-0346-1.06/14)).

Author contributions

LDCS: Conceptualization, methodology, software, formal analysis, investigation, data Curation, writing - original draft. KSF: conceptualization, methodology, formal analysis, writing - review and editing, supervision, project administration. AMSSB: conceptualization, methodology, formal analysis, writing - review and editing, supervision, coordination of infrared analysis. YPA: Methodology and investigation. JFNS: Methodology and investigation. LVPA: Methodology and investigation. ANS: conceptualization, methodology, writing - review and editing, visualization, supervision, project administration, funding acquisition.

References

Acevedo, E., Badilla, I., and Nobel, P. S. 1983. Water Relations, Diurnal Acidity Changes, and Productivity of a Cultivated Cactus, *Opuntia ficus-indica*. *Plant Physiology*. *72*(3): 775–780. https://doi.org/10.1104/PP.72.3.775.

Alencar, B. A. R., Duntra, E. D., Sampaio, E. V. D. S. B., Menezes, R. S. C., and Morais Jr, M. A. 2018. Bioresource Technology Enzymatic hydrolysis of cactus pear varieties with high solids loading for bioethanol production. *Bioresource Technology*. *250*: 273–280. ttps://doi.org/10.1016/j.biortech.2017.11.042.

Ammar, I., Ben Salem, M., Harrabi, B., Mzid, M., Bardaa, S., Sahnoun, Z., Attia, H., and Ennouri, M. 2018. Anti-inflammatory activity and phenolic composition of prickly pear (*Opuntia ficus-indica)* flowers. *Industrial Crops and Products*. *112*: 313–319.

https://doi.org/10.1016/j.indcrop.2017.12.028.

Assis, O. B. G., and Britto, D. de, 2014. Revisão: coberturas comestíveis protetoras em frutas: fundamentos e aplicações. *Brazilian Journal of Food Technology*. 17: 87–97. https://doi.org/10.1590/bjft.2014.019.

Astello-García, M. G., Cervantes, I., Nair, V., Santos-Díaz, M. del S., Reyes-Agüero, A., Guéraud, F., Negre-Salvayre, A., Rossignol, M., Cisneros-Zevallos, L., and Barba de la Rosa, A. P. 2015. Chemical composition and phenolic compounds profile of cladodes from *Opuntia* spp. cultivars with different domestication gradient. *Journal of Food Composition and Analysis*. *43*: 119–130. https://doi.org/10.1016/j.jfca.2015.04.016.

Beck, H. E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., and Wood, E. F. 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data. 5*(1): 1–12. https://doi.org/10.1038/sdata.2018.214.

Bertan, L. C., Tanada-Palmu, P. S., Siani, A. C., and Grosso, C. R. F. 2005. Effect of fatty acids and "Brazilian elemi" on composite films based on gelatin. *Food Hydrocolloids*. *19*(1): 73–82. https://doi.org/10.1016/j.foodhyd.2004.04.017.

Bradford, M. M. 1976. Determinación de proteínas: método de bradford. *Analytical Biochemistry*.

Del-Valle, V., Hernández-Muñoz, P., Guarda, A., and Galotto, M. J. 2005. Development of a cactus-mucilage edible coating (*Opuntia ficus indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf-life. *Food Chemistry*. *91*(4): 751–756. https://doi.org/10.1016/j.foodchem.2004.07.002.

Di Lorenzo, F., Silipo, A., Molinaro, A., Parrilli, M., Schiraldi, C., D'Agostino, A., Izzo, E., Rizza, L., Bonina, A., Bonina, F., and Lanzetta, R. 2017. The polysaccharide and low molecular weight components of *Opuntia ficus indica* cladodes: Structure and skin repairing properties. *Carbohydrate Polymers*. *157*: 128–136. https://doi.org/10.1016/j.carbpol.2016.09.073.

Dick, M., Limberger, C., Cruz Silveira Thys, R., de Oliveira Rios, A., and Hickmann Flôres, S. 2020. Mucilage and cladode flour from cactus (*Opuntia monacantha*) as alternative ingredients in gluten-free crackers. *Food Chemistry*. *314*: 126178. https://doi.org/10.1016/j.foodchem.2020.126178.

Du Toit, A., de Wit, M., Fouché, H. J., Venter, S. L., and Hugo, A. 2020. Relationship between weather conditions and the physicochemical characteristics of cladodes and mucilage from two cactus pear species. *PLOS ONE*. *15*(8): e0237517. https://doi.org/10.1371/JOURNAL.PONE.0237517.

Du Toit, A., De Wit, M., and Hugo, A. 2018. Cultivar and harvest month influence the nutrient content of *Opuntia* spp. Cactus pear cladode mucilage extracts. *Molecules*. 23(4): 1–12. https://doi.org/10.3390/molecules23040916.

Du Toit, A., De-Wit, M., Fouché, H. J., Taljaard, M., Venter, S. L., and Hugo, A. 2019. Mucilage powder from cactus pears as functional ingredient: influence of cultivar and harvest month on the physicochemical and technological properties. *Journal of Food Science and Technology*. *56*(5): 2404–2416 https://doi.org/10.1007/s13197-019-03706-9.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric Method for

Determination of Sugars and Related Substances. *Analytical Chemistry*. https://doi.org/10.1021/ac60111a017.

Feugang, J. M. 2006. Nutritional and medicinal use of Cactus pear *(Opuntia spp .)* cladodes and fruits. *Frontiers in Bioscience*. https://doi.org/10.2741/1992.

Figueroa-Pérez, M. G., Pérez-Ramírez, I. F., Paredes-López, O., Mondragón-Jacobo, C., and Reynoso-Camacho, R. 2018. Phytochemical Composition and in Vitro Analysis of Nopal (*O. Ficus-Indica*) Cladodes at Different Stages of Maturity. *2 International Journal of Food Properties, 21(1), 1728-1742.* https://doi.org/10.1080/10942912.2016.1206126

Gheribi, R., and Khwaldia, K. 2019. Cactus mucilage for food packaging applications. *Coatings*. *9*(10): 1–19. https://doi.org/10.3390/coatings9100655.

Gheribi, R., Puchot, L., Verge, P., Jaoued-Grayaa, N., Mezni, M., Habibi, Y., and Khwaldia, K. 2018. Development of plasticized edible films from *Opuntia ficus-indica* mucilage: A comparative study of various polyol plasticizers. *Carbohydrate Polymers*. *190:* 204-211. https://doi.org/10.1016/j.carbpol.2018.02.085.

Jaramillo-Flores, M. E., González-Cruz, L., Cornejo-Mazón, M., Dorantes-álvarez, L., Gutiérrez-López, G. F., and Hernández-Sánchez, H. 2003. Effect of Thermal Treatment on the Antioxidant Activity and Content of Carotenoids and Phenolic Compounds of Cactus Pear Cladodes (*Opuntia ficus-indica*). *Food Chemistry.* *9*(4): 271-278. https://doi.org/10.1177/108201303036093.

Liguori, G., Gentile, C., Gaglio, R., Perrone, A., Guarcello, R., Francesca, N., Fretto, S., Inglese, P., and Settanni, L. 2020. Effect of addition of *Opuntia ficus-indica* mucilage on the biological leavening, physical, nutritional, antioxidant and sensory aspects of bread. *Journal of Bioscience and Bioengineering*. *129*(2): 184–191. https://doi.org/10.1016/j.jbiosc.2019.08.009.

Lüttge, U. 2010. Ability of crassulacean acid metabolism plants to overcome interacting stresses in tropical environments. *AoB PLANTS*. *2010*. https://doi.org/10.1093/AOBPLA/PLQ005.

Lutz, A. 2008. Métodos fisico-quimicos para analise de alimentos edição IV. In *Métodos físicos-quimicos para análise de Alimentos*.

Manhivi, V. E., Venter, S., Amonsou, E. O., and Kudanga, T. 2018. Composition, thermal and rheological properties of polysaccharides from amadumbe (*Colocasia esculenta*) and cactus (*Opuntia* spp.). *Carbohydrate Polymers*. *195*: 163–169. https://doi.org/10.1016/J.CARBPOL.2018.04.062.

Males, J., and Griffiths, H. 2017. Stomatal Biology of CAM Plants. *Plant Physiology*. *174*(2): 550–560. https://doi.org/10.1104/PP.17.00114.

Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., and Katthain, R. 2000. Rheological properties of the mucilage gum (*Opuntia ficus indica*). *Food Hydrocolloids*. *14*(5): 417–424. https://doi.org/10.1016/S0268-005X(00)00015-1.

Monrroy, M., García, E., Ríos, K., and García, J. R. 2017. Extraction and Physicochemical Characterization of Mucilage from *Opuntia cochenillifera* (L.) Miller. *Journal of Chemistry*. *2017*. https://doi.org/10.1155/2017/4301901.

Morais, M. A. dos S., Fonseca, K. S., Viégas, E. K. D., de Almeida, S. L., Maia, R. K. M., Silva, V. N. S., and Simões, A. do N. 2019. Mucilage of spineless cactus in the composition of an edible coating for minimally processed yam (*Dioscorea* spp.). *Journal of Food Measurement and Characterization*. *13*(3): 2000–2008. https://doi.org/10.1007/s11694-019-00120-9.

Nabil, B., Ouaabou, R., Ouhammou, M., Saadouni, L., and Mahrouz, M. 2020. Impact of particle size on functional, physicochemical properties and antioxidant activity of cladode powder (*Opuntia ficus-indica*). *Journal of Food Science and Technology*. *57*(3): 943–954. https://doi.org/10.1007/s13197-019-04127-4.

Nharingo, T., and Moyo, M. 2016. Application of *Opuntia ficus-indica* in bioremediation of wastewaters. A critical review. *Journal of Environmental Management*. *166*: 55–72. https://doi.org/10.1016/j.jenvman.2015.10.005.

Nunes, C. S. 2011. Usos E Aplicações Da Palma Forrageira Como Uma Grande Uses and Applications Palm Forage As a Major Source of Economy for the Semiarid. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*. *6*(1): 58–66.

Panta-Araújo, Y., Brito, Andréa Monteiro Fonseca, K. S., Souza, J. F. do N., Sousa, L. D. C. de, Silva, T. G. F. da, Eugênio, D. da S., and Simões, A. do N. 2021. Characterization of mucilage from clones of *Opuntia* and *Nopalea* prickly pear cactus harvested in different seasons in Brazilian semiarid. J*ournal of the Professional Association for Cactus Developmen*t. *23*: 180–198.

Petera, B., Delattre, C., Pierre, G., Wadouachi, A., Elboutachfaiti, R., Engel, E., Poughon, L., Michaud, P., and Fenoradosoa, T. A. 2015. Characterization of arabinogalactan-rich mucilage from *Cereus triangularis* cladodes. *Carbohydrate Polymers*. *127*: 372–380. https://doi.org/10.1016/J.CARBPOL.2015.04.001.

Ribeiro, E. M. de Oliveira, Henrique da Silva, N., de Lima Filho, J. L., de Brito, J. Z., and Carvalho da Silva, M. da P. 2010. Study of carbohydrates present in the cladodes of *Opuntia ficus-indica* (fodder palm), according to age and season. *Ciência e Tecnologia de Alimentos*. *30*(4): 933–939. https://doi.org/10.1590/S0101-20612010000400015.

Rodriguez-Felix, A., and Cantwell, M. 1988. Developmental changes in composition and quality of prickly pear cactus cladodes (nopalitos). *Plant Foods for Human Nutrition*. *38*(1): 83–93. https://doi.org/10.1007/BF01092314.

Rodríguez-González, S., Martínez-Flores, H. E., Chávez-Moreno, C. K., Macías-Rodríguez, L. I., Zavala-Mendoza, E., Garnica-Romo, M. G., and Chacõn-García, L. 2014. Extraction and characterization of mucilage from wild species of *Opuntia*. *Journal of Food Process Engineering*. *37*(3): 285–292. https://doi.org/10.1111/jfpe.12084.

Sáenz, C., Sepúlveda, E., and Matsuhiro, B. 2004. *Opuntia* spp. mucilage's: A functional component with industrial perspectives. *Journal of Arid Environments*. *57*(3): 275–290. https://doi.org/10.1016/S0140-1963(03)00106-X.

Scalisi, A., Morandi, B., Inglese, P., and Lo Bianco, R. 2016. Cladode growth dynamics in *Opuntia ficus-indica* under drought. *Environmental and Experimental Botany*. *122*: 158–167. https://doi.org/10.1016/j.envexpbot.2015.10.003.

Sandoval, D. C. G., Sosa, B. L., Martínez-Ávila, G. C. G., Fuentes, H. R., Avendaño-Abarca, V. H.,

and Rojas, R. 2019. Formulation and Characterization of Edible Films Based on Organic Mucilage from Mexican *Opuntia ficus-indica*. *Coatings.* 9: 506. https://doi.org/10.3390/coatings9080506.

Sepúlveda, E., Sáenz, C., Aliaga, E., and Aceituno, C. 2007. Extraction and characterization of mucilage in *Opuntia* spp. *Journal of Arid Environments*. *68*(4): 534–545. https://doi.org/10.1016/j.jaridenv.2006.08.001.

Silva, A. P. G. da, Souza, C. C. E. de, Ribeiro, J. E. S., Santos, M. C. G. dos, Pontes, A. L. de S., and Madruga, M. S. 2015. Características Físicas, Químicas E Bromatológicas De Palma Gigante (*Opuntia ficus-indica*) E Miúda (*Nopalea cochenillifera*) Oriundas Do Estado Da Paraíba. *Revista Brasileira de Tecnologia Agroindustrial*. *9*(2). https://doi.org/10.3895/rbta.v9n2.1616.

Snyman, H. A. 2006. A greenhouse study on root dynamics of cactus pears, *Opuntia ficus-indica* and *O. robusta. Journal of Arid Environments*. *65*(4): 529–542. https://doi.org/10.1016/J.JARIDENV.2005.10.004.

Van Krevelen, D.W. 1997. Polymer Properties. *Properties of Polymers*. https://doi.org/10.1016/b978-0-444-82877-4.50008-1.

Ventol, L., Vendrell, M., Giraldez, P., and Merino, L. 2011. Traditional organic additives improve lime mortars: New old materials for restoration and building natural stone fabrics. *Construction and Building Materials*, *25*(8), 3313–3318. https://doi.org/10.1016/j.conbuildmat.2011.03.020.