Characterization of mucilage from clones of *Opuntia* and *Nopalea* prickly pear cactus harvested in different seasons in Brazilian semiarid

Yara Panta de Araújo[1], Andréa Monteiro Santana Silva Brito[1], Kelem Silva Fonseca[1], Jheizon Feitoza do Nascimento Souza[1], Lady Daiane Costa de Sousa[1], Thieres George Freire da Silva[1], Danielle da Silva Eugênio[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: : E-mail: adriano.simoes@ufrpe.br

ABSTRACT

This study proposes to characterize hydrated and refrigerated mucilage obtained from cladodes of clones of prickly pear cactus harvested during the drizzle and dry seasons in the semiarid of Brazil. Cladodes of *Opuntia stricta* [Haw.] Haw (Orelha de Elefante Mexicana [OEM] clone) and *Nopalea cochenillifera* Salm Dyck (IPA Sertânia [IPA] and Miúda [MIU] clones) were harvested at 6 am and extracted mucilage. The main bands in the infrared region were characterized. Physicochemical analyses were performed on day zero and at 12 days. Cladodes harvested in the dry season showed higher mucilage yield and soluble solid, total soluble carbohydrate, and K^+, for the three clones. The OEM clone also exhibited significant increases in pH, Na^+, and electrical conductivity when harvested in the dry season than in the wet season. In addition, the mucilage extracted from the *Opuntia* cladodes did not have changes in the carbohydrate, titratable acidity, or total soluble protein levels. The IPA and MIU clones, in turn, were characterized by parameters that remained stable during conservation (phenolic compounds, titratable acidity, K^+ and Na^+). The spectroscopic profile was similar for all studied clones. The principal component analysis allowed the formation of clusters between seasons and conservation times. It is suggested that the cladodes of the genus *Nopalea* showed better potential in the manufacture of edible films and coatings. The cladodes of the genus Opuntia, for the use of mucilage as ingredients in foods such as bread, pasta, and others, making them with better functional properties. Therefore, these factors should be considered for the use of mucilage in the industry.

Keywords: *Nopalea cochenillifera*; *Opuntia stricta*; mucilage; infrared; principal component analysis (PCA); conservation.

INTRODUCTION

The prickly pear cactus is found in several regions of the world (e.g. Mexico, Tunisia, Brazil, and Ethiopia), with a total area of about 4.5 million hectares. This plant is grown for human consumption (fruit and cladodes), for use in animal feed, and the production of dyes (Nazareno, 2017; Ochoa, M. J. & Barbera, 2017). In Brazil, the prickly pear cactus genera *Nopalea* and *Opuntia* are used almost exclusively in animal feed, especially during periods of drought due to their tolerance to low water availability and high-energy content (Melo et al., 2009; Queiroz et al., 2015). However, a few studies have looked into the use of this plant for human consumption. Research can be found investigating the fruit (De Souza et al., 2007), by-products (e.g. sweets, juices, jams) (Moura et al., 2009), and minimally processed sprouts of cactus (Araújo et al., 2018; Pereira et al., 2013). At present, prickly pear cactus biomass is considered a valuable raw material for biomolecules with applications in the packaging (Gheribi & Khwaldia, 2019).

The development of new biomaterials from agricultural by-products and wastes is not only a worldwide trend but also one of the main challenges for sustainability, through the adoption of ecological products (Mirabella et al., 2014; Youssef et al., 2015). In this respect, the mucilage extracted from the cladodes of prickly pear cactus has been applied in the food, cosmetic and pharmaceutical industries (Ammar et al., 2018; Park et al., 2001), as an emulsifying and stabilizing agent (Quinzio et al., 2018). In the food industry, it is used for the production of edible coatings and films (Allegra et al., 2016; Del-Valle et al., 2005; Morais et al., 2019). Recently, this mucilage has also been applied in the formulation of cookies (Dick et al., 2020) and bread (Liguori et al., 2020), betaxanthin encapsulation (Otálora et al., 2018), and zeaxanthin nanoencapsulation (Campo et al., 2018). Cladodes are also known to be used in medicine, with antioxidant and anti-inflammatory properties, among others (Feugang et al., 2006).

The mucilage is a hydrocolloid found in the cladodes and fruits of the cactus (Sáenz et al., 1998). It is composed of sugars, the most abundant of which are arabinose, galactose, rhamnose and xylose (Sepúlveda et al., 2007). The substance is characterized by high viscosity (Dick et al., 2019), and water-retaining (Sáenz et al., 2004), emulsifying (Dick et al., 2019), and elastic properties (Medina-Torres et al., 2000). These attributes are important in the development of edible films that are effective in preserving food during storage (Medina-Torres et al., 2000). Cladodes also have nutrients such as vitamins, minerals and functional compounds (i.e. phenolic antioxidants) (Stintzing & Carle, 2005) as well as high antioxidant capacity (Nabil et al., 2019), which make them a strong candidate for incorporation into food products. The mucilage extracted from the cladodes of prickly pear cactus is a natural and low-cost ingredient (Du Toit et al., 2018).

The vast majority of studies on prickly pear cactus mucilage and its applications in the food industry have been conducted with species of the genus *Opuntia* (Gheribi & Khwaldia, 2019). Du Toit et al. (2020) is the most recent research on the physicochemical characterization of mucilage according to climatic conditions. In addition, cultivars and harvest months of the year also influence the quality of mucilage and its technological properties (Du Toit et al., 2020; Du Toit et al., 2018) However, the cactus used was typical of South Africa, genus *Opuntia*. No studies, however, have reported the physical-chemical stability of the mucilage. In addition, there are few studies found in the literature to

characterize mucilage of the genus *Nopalea* spp. for application in the food industry, possibly due to the difficulties of this study, as it is known that due to the cactaceae presented crassulacean acid metabolism (CAM) an active composition changes according to hours (Rodriguez-Felix & Cantwell, 1988).

The physicochemical attributes of prickly pear cactus mucilage can also change depending on the extraction process, raw material used (Rodríguez-González et al., 2014), particle size (Kaewmanee et al., 2014), age and season of the year (Ribeiro et al., 2010). These properties can alter the edible biofilms produced from the mucilage of different clones (Sandoval et al., 2019). Because of this, a more in-depth study is warranted to elucidate the mucilage yield potential of other clones of the genera *Opuntia* and *Nopalea* like the effect of the season of the year on the physicochemical and biochemical composition of hydrated and refrigerated mucilage.

Therefore, the present study aimed to characterize the hydrated and refrigerated mucilage from cladodes of clones of prickly pear cactus of the genera *Opuntia* and *Nopalea* harvested during the wet and dry seasons of the semi-arid region of Brazil.

MATERIAL AND METHODS

Cladode collection area and experimental design in the laboratory

The cactus cladodes were collected in a growing area of the International Reference Center for Agrometeorological Studies of Cactus and other Forage Plants, in the municipality of Serra Talhada, PE, Brazil (7º59 'S; 38º15' W and 431 m). According to the Köppen classification system, the climate of the region is a BShw' type (Alvares et al., 2013). The average annual precipitation is 642 mm, average air temperature is 24.8 ºC, relative humidity is 62% and atmospheric demand for water is above 1,800 mm per year (Pereira et al., 2015). The soil in the growing area were analyzed and classified as a typic eutric Haplic Cambisol Ta, with the following chemical properties: pH (H_2O) = 5.95; CE_e = 0.32 dS m^{-1}; P = 168.96 mg dm^{-3}; K^+ = 13.8 cmolc dm^{-3}; Na^+ = 1.09 cmolc dm^{-3}; Ca^{2+} = 3.45 cmolc dm^{-3}; Mg^{2+} = 1.90 cmolc dm^{-3}; H + Al = 0.6 cmolc dm^{-3}; sum of bases = 20.25 cmolc dm^{-3}; cation-exchange capacity = 20.85 cmolc dm^{-3}; base saturation = 97.15%; organic carbon = 4.6 g kg^{-1}; and organic matter = 7.93 g kg^{-1}. Physical properties are as follows: sand = 828.6 g kg^{-1}; silt = 148.25 g kg^{-1}; clay = 23.15 g kg^{-1}; and soil density = 1.45 g dm^{-3}.

The growing area was established with the IPA Sertânia (‘IPA’; *Nopalea cochenillifera* Salm Dyck), Miúda (‘MIU’; *Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (‘OEM’; *Opuntia stricta* [Haw.] Haw.) clones in February 2016. The cladodes were inserted vertically in the soil at a spacing of 1.0 × 0.2 m, which resulted in a stand of 50,000 plants ha^{-1}. Fertilization were carried out based on soil analysis, which resulted in the equivalent application of 73.5 kg N ha^{-1}, 94.5 kg K_2O ha^{-1} and 84 kg S ha^{-1}. Cleaning ways were performed when was necessary. No phytosanitary treatment was necessary.

The cladodes of the three clones were collected in two seasons wet and dry and harvested always at 6 am (maximum time of two hours). In the wet season, the cladodes were collected throughout May 2019, and in the dry season, in November 2019. Figure 1 illustrates the weather conditions throughout the cladode collection period. Cladodes 100 to 240 mm long were selected for ‘MIU’ and 240 to 300 mm long for ‘IPA’ and ‘OEM’. In the

laboratory, after extracting the mucilage and obtaining the powder, two storage times were considered, namely, 0 and 12 days. The experiment was laid out in a completely randomized design for each clone analyzed, considering the two harvest seasons and two conservation times, in four replicates.

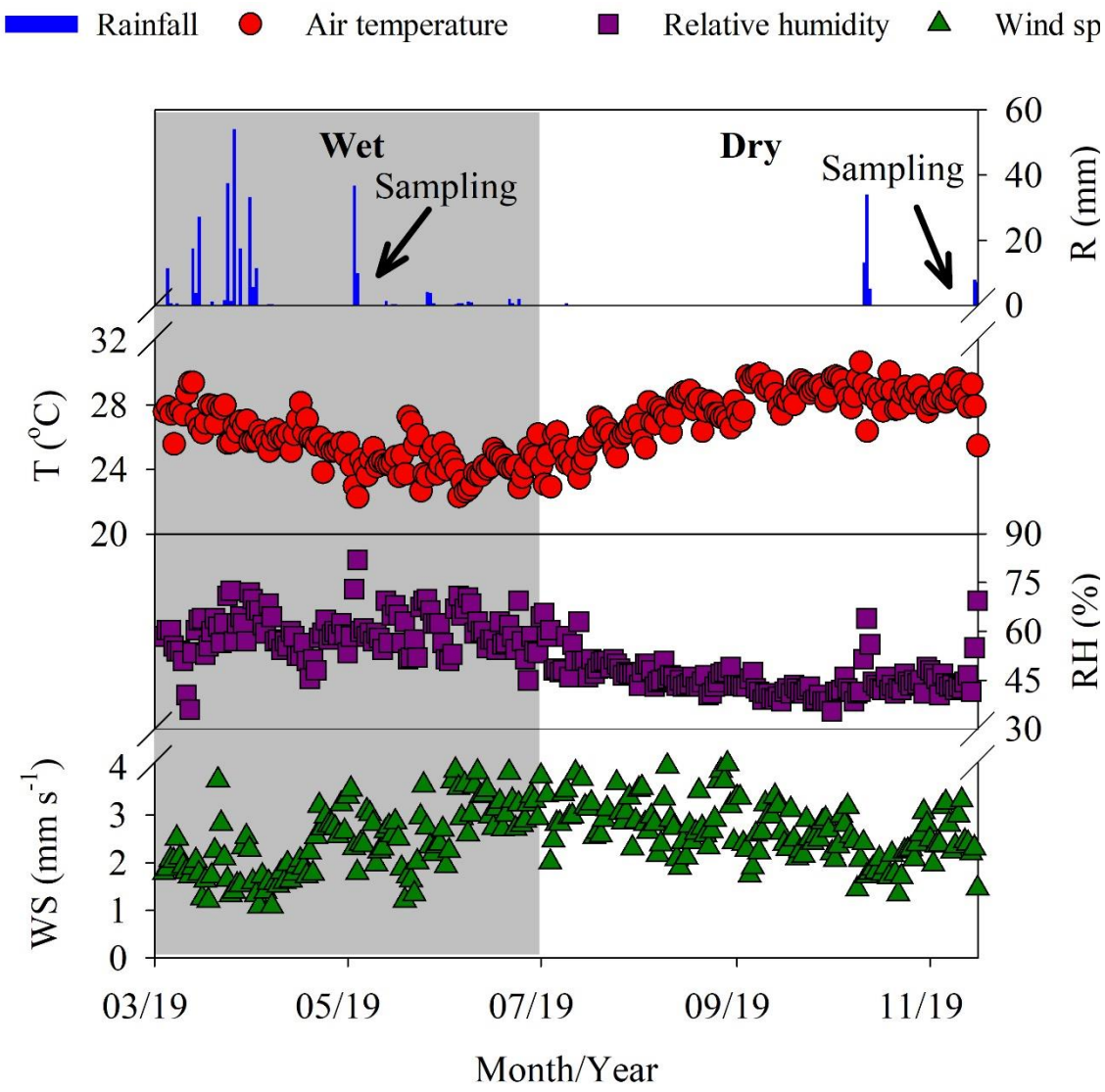

Figure 1. Precipitation (mm), air temperature (°C), relative humidity (%) and wind speed (mm s^{-1}) from March to June 2019 (wet season) and July to November 2019 (dry season) in the municipality of Serra Talhada-PE, Brazil. Source: Instituto Nacional de Meteorologia (INMET), 2020.

Mucilage powder production and mucilage yield

The mucilage was extracted by a modified version of the method proposed by Gheribi et al. (2018). The cladodes were weighed and washed under running water; the epidermis was removed with knives and the resulting parenchyma was used to extract the mucilage. The parenchyma was weighed and ground in a food processor (ri7775, Philips Walita, Barueri, Brazil). Subsequently, ethanol (99.8%) was added and the material was homogenized and washed twice to remove the pigments. The precipitated material was dried in a forced-air oven at 55 °C for 48 h. Afterward, the dry powder was pulverized using a portable mill

(Polespresso, Original coffee flavor, Carapin da Serra, Brazil) and kept at 26 °C in a display case.

The mucilage yield was obtained from the fresh weight of the whole cladodes and the weight of the powdered mucilage, using the following formula:

$$MY = \frac{W_f}{W_i}100 \quad (1)$$

Where *MY* is the mucilage yield in percentage values (%), on a fresh-weight basis; W_f is the final weight of the powdered mucilage (g), and; W_i is the initial weight of the whole cladodes (g).

Mucilage hydration

The mucilage powder was hydrated using a food processor, for 1 min, in the proportion of 4% (w w^{-1}) Gheribi et al. (2018). The hydrated mucilage was kept at 5 °C for 12 days. Physicochemical and biochemical analyses were performed at the beginning of the experiment, on day 0, and after 12 days of refrigerated storage.

Soluble solids (SS) and total soluble carbohydrates (TC)

The soluble solids content of the mucilage was measured using a bench refractometer (Instrutherm, RTD-95, São Paulo, Brazil). Readings were performed using 0.5 mL of hydrated mucilage. Results were expressed in °Brix.

Total soluble carbohydrates were obtained by a modified version of the method proposed by Dubois et al. (1956). The hydrated mucilage (2 mL) was 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 deionized water, 500 µL phenol (5%) and 2500 µL sulfuric acid (AR grade). The tubes were vortexed (TECNAL, AP56, Araraquara, Brazil) and kept at rest for 10 min. Readings were taken with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 490 nm and the total carbohydrate content was expressed in g of soluble carbohydrates per 100 g DW.

pH, titratable acidity (TA) and vitamin C content (vit. C)

The pH was determined using a pH meter (TECNAL, TEC-5, Piracicaba, Brazil), at a temperature of 25 °C, by immersing the electrode directly into the hydrated mucilage samples (IAL, 2008).

Titratable acidity was determined by a modified version of the procedures suggested by Astello-García et al. (2015). The hydrated mucilage was titrated with aqueous sodium hydroxide solution (NaOH) 0.1 N. The titratable acidity was calculated by the following formula:

$$\mathrm{TA} = \frac{(\mathrm{NVEq}_{\mathrm{citric\ acid}})}{\mathrm{v}} \quad (2)$$

Where *TA* = titratable acidity; *N* = NaOH concentration; *V* = volume of NaOH used in titration (mL); *Eq* = gram-equivalent of citric acid (64.02); and *v* = sample volume (mL). Results were expressed in % citric acid.

The vitamin C content was determined by titration, using Tillman's solution, following the method described by (IAL, 2008). For this: a 10 mL aliquot of hydrated mucilage-containing acid solution was used. The standard was prepared with a solution of vit. C, acid solution and water, whereas the reference solution was prepared using water and acid solution. The following equation was applied:

$$vit\ C = \frac{(VF100)}{S} \qquad (3)$$

Where *V* = volume of Tillmans solution used in the titration (mL); *F* = Tillmans solution correction factor; and *S* = sample volume (mL). Results are expressing on mg of ascorbic acid per 100 g DW.

Sodium content (Na^+), potassium content (K^+) and electrical conductivity (EC)

Na^+ and K^+ contents were obtained with a flame photometer (Micronal, B462, Piracicaba, Brazil), using a final volume of 15 mL, at the hydrated mucilage: deionized water ratio of 1:50. Results were expressed in mg of K^+ or Na^+ per 100 g DW.

Electrical conductivity was determined with a benchtop conductivity meter (TECNAL, Tec-4MP, Piracicaba, Brazil), by immersing the electrode directly into the hydrated mucilage samples. Results were expressed in mS cm^{-1}.

Total phenolic compounds (TPC) and total soluble proteins (TSP)

The total phenolic compound contents were determined by a modified version of the method described by Jaramillo-Flores et al. (2003). A 2 mL volume of hydrated mucilage was placed 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 then mixed 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. Readings were taken with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 725 nm. The TPC content was expressed in mg of gallic acid per 100 g DW.

The total soluble protein content was determined according to Bradford, (1976), with 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. Then, 100 µL of the supernatant was added to 1000 µL of Bradford reagent. The tubes were vortexed (Tecnal, AP56, Araraquara, Brazil) and remained at room temperature for 15 min. Readings were taken using a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 595 nm. Bovine serum albumin (BSA), assuming it was 100% pure, was used as an external standard. The TSP content was expressed in mg of soluble protein per 100 g DW.

Fourier transform infrared spectroscopy (FTIR)

Spectral analyses in the mid-infrared region were conducted in a Fourier transform infrared spectrophotometer (FTIR) (Perkin Elmer® Frontier), using the universal attenuated total reflectance (UATR) accessory. The spectra were acquired in the area of 4000-400 cm^{-1}, fewer than 8 cm^{-1} resolutions, with eight scans. Air was used as the blank and measurements were taken in quadruplicate, directly on the mucilage powder under the crystal. The FTIR analysis was performed on powder samples only on day 0, to characterize the functional groups of powdered mucilage. This analysis weren´t performed on hydrated mucilage to prevent the water from interfering with the functional groups characteristic of mucilage.

Statistical analysis

Shapiro-Wilk's test is applied for normality of residuals and Levene's test for homogeneity between variances. When these two assumptions were met, analyses of variance (ANOVA), with two factors, were used for the physicochemical and biochemical data, at the 5% significance level, by Fisher-Snedecor's F-test. Insignificant cases, means were compared by Tukey's test, at 5% significance. For these analyses, SAS software was used (SAS Software, 1996). For principal components analysis (PCA), the XLSTAT (Addinsoft, 2020) software tool was used in which the means of the physicochemical, biochemical, and FTIR integrated data were decomposed into sets of orthogonal vectors. The results of the correlation matrix were displayed in biplots with their distribution in the space of orderings, variances, and Pearson's correlation. The graphs were created using SigmaPlot software version 14 (Systat Software Inc., 2020).

RESULTS AND DISCUSSION

In this study, we examined the yields and some important physicochemical properties in the characterization of mucilage for the food industry, using the material extracted from cladodes of prickly pear cactus immediately after hydration (i.e. on day zero) and after 12 days under storage at 5 °C.

The mucilage yields from the cladodes of the IPA Sertânia ('IPA'), Miúda ('MIU') e Orelha de Elefante Mexicana ('OEM') was 1.5, 2.4 e 1.1% in the wet season, respectively. In the season dry was 5.0, 7.3 e 2.3 for 'IPA', 'MIU', and 'OEM', respectively. The mucilage yields obtained from the cladodes of the 'IPA', 'MIU', and 'OEM' clones were higher in the dry season than in the wet season. Furthermore, the mucilage yield of 'MIU' was higher than those of the two other clones. Thus, it was noticed that the hotter and drier periods provided greater yields in mucilage, as also proposed by Du Toit et al. (2020). This can be explained due to the structural change in the interaction between water and the charged mucilage structure (Du Toit et al., 2020). Therefore, in these harvest conditions, new opportunities can be created for farmers in semi-arid regions, producing raw material that allows for multiple uses of cladodes, such as in the food and packaging industry.

The wet season had total accumulated precipitation of 285 mm, an average air temperature of 25 °C and 59% relative humidity (Fig. 1). In the dry season, total accumulated precipitation was 68 mm, the average air temperature was 28 °C and relative humidity was 43% (Fig. 1). These climatic differences resulted in significant differences in mucilage yield.

The yields of all studied clones in the dry season were higher than those found by Cárdenas et al. (1997) (0.07% fresh weight in *Opuntia ficus indica*), Sepúlveda et al. (2007) (1.33% fresh weight in *Opuntia* spp.), and Dick et al. (2019) (1.2% fresh weight in *Opuntia monocantha*). In addition, environmental fluctuations modulated the physicochemical parameters of the mucilage, as indicated in the PCAs (Fig. 7). The mucilage obtained from cladodes harvested in the dry season exhibited significantly higher SS, TC, and K^+ values in all studied clones (Fig. 2; Fig. 4 A, B, and C).

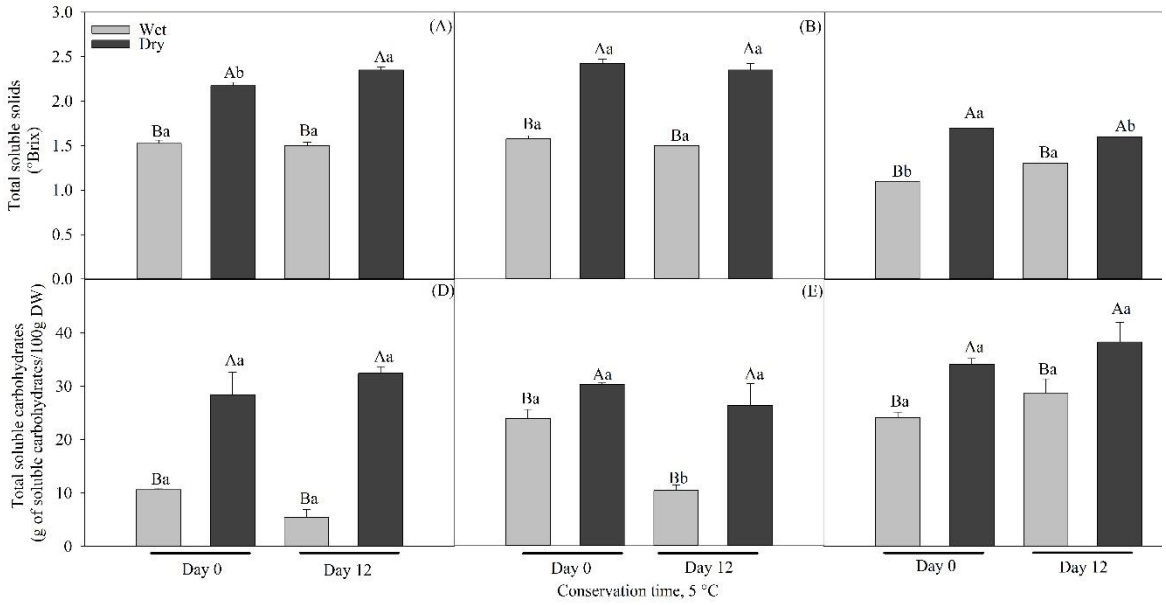

Figure 2. Soluble solids and total carbohydrate contents in mucilage extracted from cladodes of prickly pear cactus (IPA, Miúda and Orelha de Elefante Mexicana) collected in the wet and dry seasons and stored for 12 days at 5 °C. Clones: IPA Sertânia (A and D), Miúda (B and E) and Orelha de Elefante Mexicana (C and F). Bars represent the standard deviation of the mean. Letters represent statistical differences between the means by Tukey's test, at 5% probability; uppercase for seasons and lowercase for storage days.

The mucilage extracted from the 'OEM' clone during the dry season also showed higher pH, Na^+ and EC values (Fig. 3 C; Fig. 4 F and I). A higher TC content in the dry season was also reported by Ribeiro et al. (2010) for the genus *Opuntia*, which may be associated with greater drought tolerance. Moreover, the cladodes harvested in the dry season were found to be less turgid than those harvested in the wet season, which was a consequence of the four times higher precipitation in the latter season. Nas plantas, o aumento de K^+ desempenha papel fundamental no crescimento e desenvolvimento vegetal, participando de processos como ativação enzimática, síntese de proteínas, abertura estomática, fotossíntese e resistência a estresses, como salinidade, frio, seca, entre outros. Neste último, o acúmulo de K^+ no citosol e no vacúolo pode promover a aquisição de água, dessa forma aumentando o ajuste osmótico de plantas (Wang et al., 2013). Nos alimentos, a ingestão de K^+ é benéfica contra doenças como hipertensão e acidente vascular cerebral, além de ser fundamental para manter o potencial de membrana das células e na regulação da função nervosa e muscular (Steffensen et al., 2018), desta forma é um nutriente essencial para o funcionamento saudável do corpo humano (Singh & Chandorkar, 2018).

In the cladodes of prickly pear cactus, dehydration occurs in the storage parenchyma, which can lose up to 82% water, without irreversible damage to the tissue (Goldstein et al., 1991). Coupled with this, there is an accumulation of carbohydrates present in the mucilage, in the intercellular spaces and in the cell wall. This maintains the water potential gradient, which ensures the movement of water to the photosynthetic tissues (Goldstein et al., 1991). In the wet season, when the cladodes are turgid, there is greatest water availability in the soil, temperatures are lower and relative humidity higher (Santos & Calesso, 1998). Intercellular spaces are thus reduced due to the large amount of water within the cells, which results in less mucilage production in the wet season.

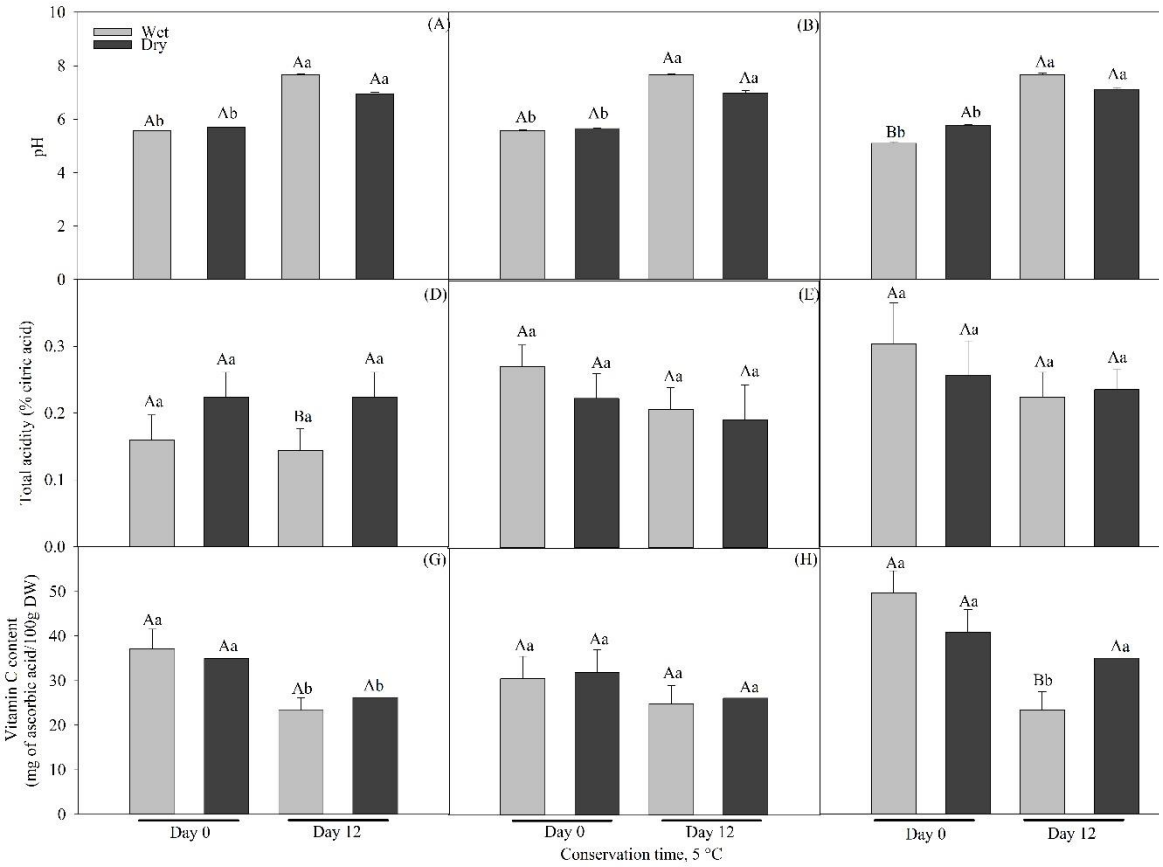

Figure 3. pH, titratable acidity and vitamin C content of mucilage extracted from cladodes of prickly pear cactus (IPA, Miúda and Orelha de Elefante Mexicana) harvested in the wet and dry seasons and stored for 12 days at 5 °C. Clones: IPA Sertânia (A; D and G), Miúda (B; E and H) and Orelha de Elefante Mexicana (C; F and I). Bars represent the standard deviation of the mean. Letters represent statistical differences between the means by Tukey's test, at 5% probability; uppercase for seasons and lowercase for storage days.

The mucilage extracted from the 'IPA' cladodes and kept at 5 ºC for 12 days maintained its TC, K^+, Na^+ and TPC levels in relation to the start of the experiment (Fig. 2 D; Fig. 4 A, D and Fig. 5 A). Conversely, for the mucilage extracted from the 'OEM' clone during the wet season, storage increased the levels of SS, TC, K^+, Na^+ and CE (Fig. 2 C and F; Fig. 4 C, F and I). These results are good indicators for the formulation of an edible coating, since fruits or vegetables require refrigeration. Increases in the concentration of electrolytes in the mucilage reduce the viscosity because it causes a break in the molecular confirmation. (Du

Toit et al., 2019) This is not desirable in the formulation of films and coatings as it reduce the ability to adhere to the surface of the coated product (Assis & Britto, 2014; Van Krevelen, 1997). In the present study, although the dry season has increased the concentration of electrolytes (Na^+, K^+ and EC), the *Nopalea* clones, represented by IPA and 'MIU', had the lowest electrolyte increments. This may be an important aspect for the selection of these clones for the production of edible films and coatings. It is known that the ideal pH for film formulation is between 5.6 and 7, because on this range there is a spreading of the molecular configuration of the mucilage, due to the reduction of the repulsion forces and a greater number of intermolecular hydrogen bonds. This results in a more orderly three-dimensional network, producing compact and resistant films (Espino-Díaz et al., 2010). In the present study, although the pH values tended to increase, they were within the range (Fig 3 A, B and C).

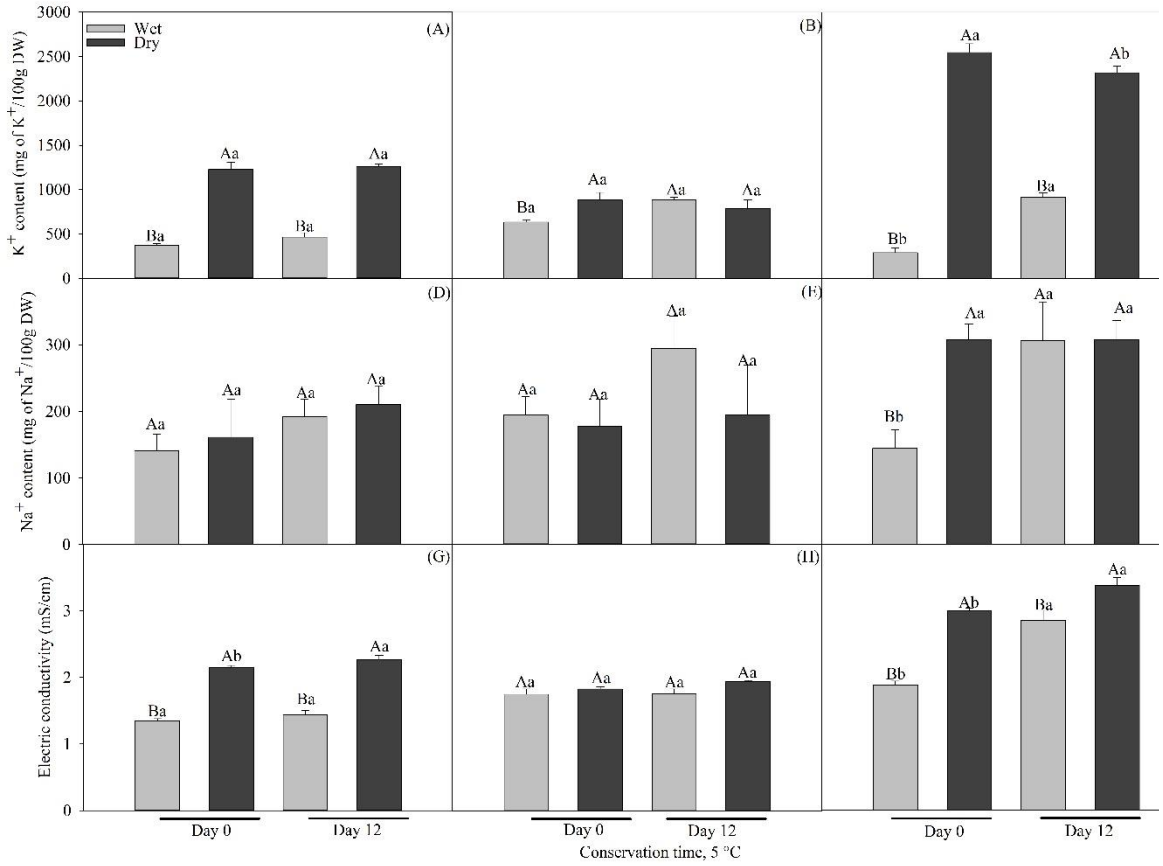

Figure 4. K+, Na+ and electrical conductivity in mucilage extracted from cladodes of prickly pear cactus (IPA, Miúda and Orelha de Elefante Mexicana clones) harvested in the wet and dry seasons and stored for 12 days at 5 °C. Clones: IPA Sertânia (A, D and G), Miúda (B, E and H) and Orelha de Elefante Mexicana (C, F and I). Bars represent the standard deviation of the mean. Letters represent statistical differences between the means by Tukey's test, at 5% probability; uppercase for seasons and lowercase for storage days.

Additionally, after 12 days, the mucilage from 'OEM', *Opuntia*, had a darker appearance than the mucilage extracted from the other two clones, of the genus *Nopalea* (Fig. 6 B). This result coincides with the highest TPC content found in this clone of the genus *Opuntia* during storage (Fig. 5 C). Phenolic compounds may favor the antioxidant activity of a foodstuff

(Nabil et al., 2019). On the other hand, the formulation of edible films is undesirable, as these compounds can react with the polysaccharides of mucilage, reducing film production and increasing the water barrier properties (Jaramillo-Flores et al., 2003).

Another characteristic of ingredients for the composition of food products is their nutritional or functional capacity (e.g. vit. C and organic acids). According to Medina-Torres et al. (2011), the vit. C content of cladodes is approximately 200 mg 100 g^{-1} (dry weight basis) and their acidity ranges between 2.0 and 4.3% citric acid (Astello-García et al., 2015). The values recorded in the present study (Fig. 3 G, H and I) were lower than those reported by Medina-Torres et al. (2011), which is explained by the mucilage extraction process with ethanol and exposure to a temperature of 55 °C for a period of 24 h. These conditions contributed to the partial degradation of vit. C and organic acids in the mucilage. The drying process can reduce the ascorbic acid content of *Opuntia* cladodes by up to 80% (Medina-Torres et al., 2011).

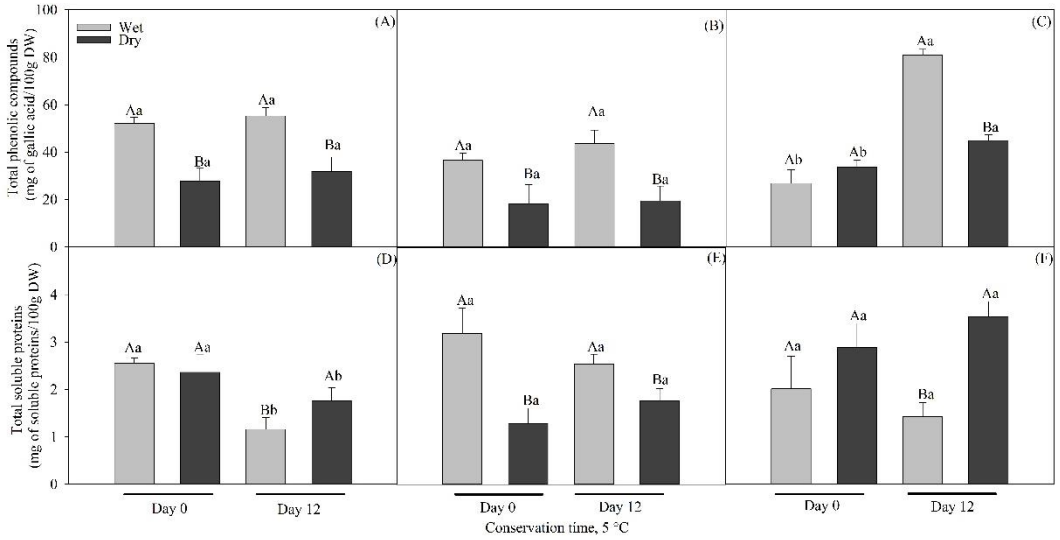

Figure 5. Total phenolic compounds and total soluble proteins in mucilage extracted from cladodes of prickly pear cactus (IPA, Miúda, and Orelha de Elefante Mexicana clones) harvested in the wet and dry seasons and stored for 12 days at 5 °C. Clones: IPA Sertânia (A, D and G), Miúda (B, E, and H) and Orelha de Elefante Mexicana (C, F, and I). Bars represent the standard deviation of the mean. Letters represent statistical differences between the means by Tukey's test, at 5% probability; uppercase for the season and lowercase for storage days.

Mucilage the prickly pear cactus is composed of a complex mixture of macromolecules with a larger portion of polysaccharides (~14% of the dry weight) (Goldstein et al., 1991), like glucose, fructose, galactose, xylose, and arabinose (Ribeiro et al., 2010). The characteristics of the main functional groups associated with the mucilage found in the three studied clones were similar to those reported in the literature (Gheribi et al., 2018; Rodríguez-González et al., 2014), verified in fig 6 A. The major bands were found at 3331 cm^{-1}, attributed to the OH stretch of alcohol, carboxylic acid, and hydrogen intermolecular bonding; and at 2926 cm^{-1}, attributed to vibrations of CH bonds, which include symmetric and asymmetric stretching of C-H, CH_2, and CH_3 bonds of molecules (Bayar et al., 2016; Bernardino-Nicanor et al., 2018; Gheribi et al., 2019; Rodríguez-González et al., 2014).

Because mucilage contains a carboxylic acid salt, the carboxylate ion (COO^-) originates two bands: a more intense one at 1620 cm^{-1}, from axial asymmetric deformation; and a weaker one at 1347 cm^{-1}, from axial symmetric deformation (Rodríguez-González et al., 2014). In addition to a set of peaks in the region between 1320 and 1240 cm^{-1} that correspond to the C-H, CH_2, and O-H vibrations, the peak of 1044 cm^{-1}, which corresponds to the C-C and C-O vibrations, is more indicative of the presence of polysaccharides in the mucilage (Gheribi et al., 2019; Rodríguez-González et al., 2014).

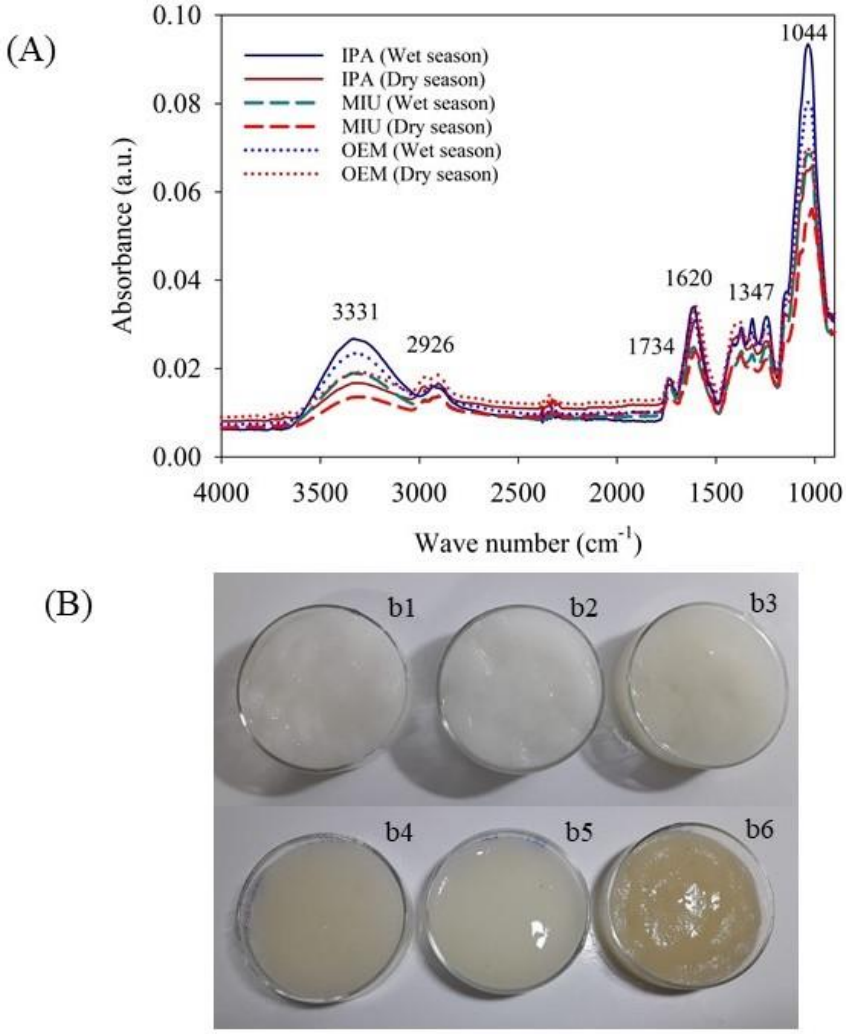

Figure 6. Medium spectra in FTIR of powdered mucilage extracted from cladodes of the IPA Sertânia (IPA), Miúda (MIU), and Orelha de Elefante Mexicana (OEM) clones were harvested in the wet and dry seasons (A). Photographs of mucilage extracted from prickly pear cactus IPA (B1 and B4), MIU (B2 and B5), and OEM (B3 and B6) harvested in the wet season, at the beginning (B1, B2, and B3) and after 12 days (B4, B5, and B6) of refrigeration (B).

Overall, in the three clones, part of the polygalacturonic acids (pectin) present in the mucilage is methoxylated, which is visible through a small peak in the region of 1734 cm^{-1} (Fig. 6 A), a characteristic of mucilage with a certain degree of esterification (Bayar et al., 2016; Rodríguez-González et al., 2014). However, it should be mentioned that, at high carbohydrate concentrations, pectins with a high or low degree of esterification can absorb

water and form a gel. Thus, although the mucilage from the clones showed a slight peak in the esterification region, these cladodes have considerable carbohydrate levels, especially when harvested in the dry season. As such, they are promising for film formation (Brandão & Andrade, 1999).

The principal component analysis allowed the study of the relationships between the physicochemical data for each group of samples (clones, seasons and conservation times) (Fig. 7). Groups were formed for the dry and wet seasons (Fig. 7 A) and there was a trend for the formation of groups between the treatments of 0 and 12 days (Fig. 7 B), for all analyzed clones. In the PCA that included storage time (0 and 12 days) and excluded the FTIR variable, 84% of the total variation of the data were explained by three principal components (PC1 = 36%, PC2 = 30% and PC3 = 18%) (Fig. 7 B). The times (0 and 12 days) were positively correlated, whereas the seasons (Wet vs. Dry) and clones (IPA vs. OEM) were negatively correlated with the Dry-OEM-12 and Dry-OEM-0 sets, which were positively explained by EC, K^+, TC and TA. Wet-IPA-12 and Wet-IPA-0, in turn, were negatively correlated with these variables. The Wet-MIU-12 and Wet-OEM-12 sets showed a positive correlation with each other, but an inverse correlation with Wet-MIU-0, Dry-IPA-0 and Wet-OEM-0. The storage time of 12 days in PC2 showed as positive association with TPC, pH and Na^+ and a negative association with vit. C. Conversely, day 0 had a positive correlation with vit. C and a negative correlation with TPC, pH and Na^+. In PC3, more groups were formed according to the season (Dry), which was explained by the TSP variable. These differences in the clusters were formed between the studied cactus genera according to harvest time and storage period reinforce the changes in the physicochemical composition of the mucilage, which may be a factor to be considered when using the material on a large scale. This can result in distinct interactions for incorporation into food products.

Cladodes harvested in the dry season increased yield and carbohydrates. This is a potential environmental modulator for producing mucilage for use in the edible film and coating industry. The cladodes of the genus *Nopalea* showed better potential in the manufacture of edible films and coatings, as they presented high levels of carbohydrates, and lower levels of Na^+, K^+ and EC. On the other hand, the cladodes of the genus Opuntia stood out with the highest levels of total phenolic compounds. This feature favors this clone for the use of mucilage as ingredients in foods such as bread, pasta, among others, making them with better functional properties. Finally, the data reveal the importance of systematically handling the raw material, as proposed by Du Toit et al. (2020). Especially with regard to the environmental conditions and the cactus clones to be used for industrial purposes.

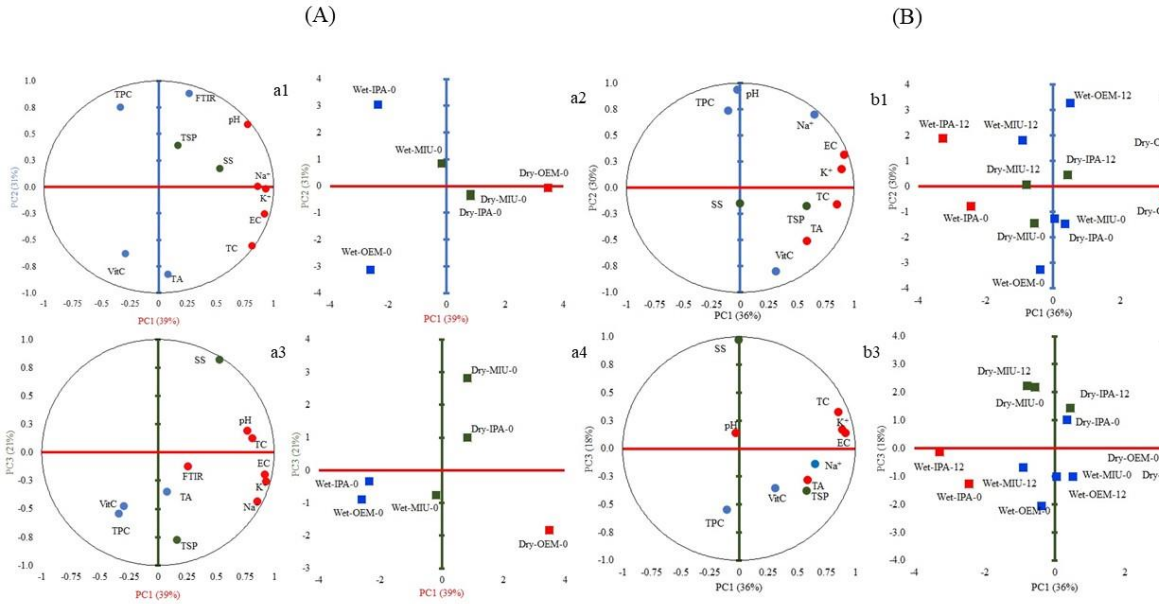

Figure 7. Scores obtained by PCA of physicochemical and biochemical data of powdered mucilage extracted from cladodes of the IPA Sertânia, Miúda, and Orelha de Elefante Mexicana clones harvested in the wet and dry seasons (A). Scores obtained by PCA of physicochemical and biochemical data of powdered mucilage extracted from cladodes of the IPA Sertânia, Miúda, and Orelha de Elefante Mexicana clones harvested in the wet and dry seasons and stored for 12 days (B).

CONCLUSIONS

This distinction between clones, seasons and storage times indicates that these factors must be taken into account for the use of mucilage in the food industry. The cactus clones harvested in the dry season exhibited a different physicochemical and biochemical composition than those grown in the wet season, which was also observed in the groups formed by PCA of physicochemical and biochemical data. Additionally, storing the hydrated mucilage at 5 ºC for 12 days resulted in an increase in the pH of all clones. Refrigerated storage did not alter the TC, TA, or TSP levels of mucilage extracted from the studied *Opuntia* cladodes. On the other hand, the mucilage from clones of the genus *Nopalea* exhibited more parameters that remained stable for 12 days (TC, K^+, Na^+ and TA), with MIU showing no significant variations in TSP, SS, Vit. C or EC over the 12 days of conservation. This change in storage was evident in the groups formed by PCA. In addition, the results indicate that the factors evaluated in the present study may enhance the use of mucilage extracted from cladodes of the genera *Nopalea* and *Opuntia* by the food industry.

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