YARA PANTA DE ARAÚJO

CARACTERIZAÇÃO FÍSICO-QUÍMICA E BIOQUÍMICA DE MUCILAGEM DE PALMA COLHIDAS EM DUAS ESTAÇÕES DO ANO NO SEMIÁRIDO BRASILEIRO

Serra Talhada-PE
2021

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Dissertação ou apresentada à Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, como parte das exigências do Programa de Pós-Graduação em Produção Vegetal, para obtenção do título de Mestre em Produção Vegetal.

Orientador: Prof. Dr. Adriano do Nascimento Simões
Coorientadora: Profa. Dra. Andréa Monteiro Santana Silva

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À minha mãe Maria Marluce Panta dos Santos que é o alicerce da minha vida, esteve sempre ao meu lado me apoiando em todas as minhas decisões, e guiando meu caminho, me ensinando a ser uma pessoa melhor, sempre com muito amor.

Dedico

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A persistência é o caminho do êxito.

(Charles Chaplin)

RESUMO GERAL

A mucilagem de palma pode ser aplicada em diversas áreas da indústria, por ser uma fonte natural de polissacarídeos. Alguns estudos já evidenciam que muitos fatores ambientais (temperatura, luz, umidade, disponibilidade hídrica, entre outros) alteram as propriedades da mucilagem de palma. Com isso, objetivou-se com este trabalho realizar uma caracterização físico-química e bioquímica da mucilagem de três clones de palma forrageira, em duas estações e sob armazenamento refrigerado durante 12 dias. O trabalho foi realizado na Unidade Acadêmica de Serra Talhada da Universidade Federal Rural de Pernambuco, em Serra Talhada-PE. A mucilagem foi extraída em etanol com auxílio de um multiprocessador, e foi seca em estufa, em seguida, hidratada e mantida a 5 °C por 12 dias. Foi quantificado o rendimento em pó e realizadas as seguintes análises no dia zero e aos 12 dias: sólidos solúveis totais, carboidratos solúveis, pH, acidez titulável, teor de vitamina C, teor de sódio e potássio, condutividade elétrica, compostos fenólicos totais, proteínas solúveis totais e espectroscopia do infravermelho por transformada de Fourier. O experimento foi realizado em delineamento inteiramente casualizado, com quatro repetições. Os dados foram submetidos à ANOVA, e quando significativo foram submetidos ao teste de Tukey, a 5% de probabilidade. Além disso, foi feita uma análise de componentes principais (PCA) a fim de observar a formação de grupamentos. Os clones de palma colhidos na estação seca exibiram uma composição físico-química e bioquímica distinta de quando cultivados na estação chuvosa, o que foi confirmado através dos grupamentos formados na PCA, além de evidenciar mudanças no armazenamento. Dessa forma, a mucilagem obtida de cladódios colhidos na estação seca obteve maior rendimento, sólidos solúveis, carboidratos solúveis totais e teor de K^+ para os três clones. O perfil espectroscópico foi similar para todos os clones estudados. Conclui-se que, a composição físico-química e bioquímica da mucilagem varia de acordo com o clone e a estação do ano, logo devem ser consideradas para o uso da mucilagem na indústria de alimentos.

Palavras-chaves: Palma forrageira. Biodegradável. Mucilagem. *Opuntia*. *Nopalea*.

GENERAL ABSTRACT

Palm mucilage can be applied in several areas of the industry, as it is a natural source of polysaccharides. Some studies already show that many environmental factors (temperature, light, humidity, water availability, among others) alter the properties of palm mucilage. Thus, the objective of this work was to carry out a physical-chemical and biochemical characterization of the mucilage of three forage palm clones, in two seasons and under cold storage for 12 days. The work was carried out at the Serra Talhada Academic Unit of the Federal Rural University of Pernambuco, in Serra Talhada-PE. The mucilage was extracted in ethanol with the aid of a multiprocessor, and was dried in an oven, then hydrated and maintained at 5 ° C for 12 days. The powder yield was quantified and performed according to the following analyzes on day zero and at 12 days: total soluble solids, soluble carbohydrates, pH, titratable acidity, vitamin C content, sodium and potassium content, electrical conductivity, total phenolic compounds, total soluble proteins and Fourier transform infrared spectroscopy. The experiment was carried out in a completely randomized design, with four replications. The data were discovered using ANOVA, and when significant, they were found on the Tukey test at 5% probability. In addition, a principal component analysis (PCA) was performed in order to observe the formation of clusters. Palm clones harvested in the dry season exhibited a different physical-chemical and biochemical composition than when grown in the rainy season, which was confirmed by the groups formed in the PCA, in addition to showing changes in storage. Thus, the mucilage obtained from cladodes harvested in the dry season has high yield, soluble solids, total soluble carbohydrates and K + content for the three clones. The spectroscopic profile is similar for all studied clones. It is concluded that, that the physical-chemical and biochemical composition of the mucilage varies according to the clone and the season, the logo must be considered for the use of mucilage in the food industry.

Keywords: Forage palm. Biodegradable. Mucilage. Opuntia. Nopal.

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APRESENTAÇÃO

A palma forrageira é uma das cactáceas mais abundantes no nordeste brasileiro, bem como é muito utilizada para alimentação animal, sendo este apenas um dos seus potenciais usos, o que se deve a sua demasiada capacidade de retenção hídrica, o que lhe atribui alta resistência a climas com temperaturas elevadas como o semiárido. Recentemente vários estudos têm sido produzidos com base neste vegetal afim de aumentar seus usos para a indústria alimentícia e agropecuária como fonte de recurso alternativo as épocas de estiagem e baixa produção alimentícia.

A mucilagem de palma é utilizada em diversas áreas da indústria. Na indústria farmacêutica esta é utilizada por possuir propriedades de cicatrização. Atua também na descontaminação de águas. Na construção civil, é utilizada para melhorar a aderência de argamassas. É utilizada na produção de bioetanol, e na medicina, no tratamento de diabetes, inflamações, doenças virais e câncer. Além disso, essa tem se destacado na indústria de alimentos. Na alimentação, é utilizada para a fabricação de pães e biscoitos em substituição ao glúten, além de ser um polímero natural muito utilizado na fabricação de filmes e revestimentos comestíveis. Mucilagens de diferentes fontes (cladódios, cascas de frutos, frutos) são compostos de grande potencial a ser aplicado em diversas áreas da indústria, por apresentarem alta viscosidade, retenção de água, propriedades emulsificantes, e propriedades elásticas, além de ser uma fonte natural e renovável de recursos.

Alguns trabalhos evidenciam que as características físico-químicas e bioquímicas da mucilagem podem mudar em função da espécie ou do clone, da idade do cladódio, do processo de extração, do horário de colheita (devido ao metabolismo CAM), do tamanho das partículas e da estação do ano. Tais propriedades podem afetar os produtos formados a partir da mucilagem extraída nas diferentes condições. Apesar dos diversos trabalhos visando a mucilagem, percebe-se uma falta de padronização quanto as condições de obtenção desse material, o que pode mascarar os dados obtidos, uma vez que, uma diversidade de fatores afeta o crescimento dos cladódios, tais como, temperatura, luminosidade, umidade do solo e do ar, turgidez celular, idade e horário de colheita, entre outros. Além disso, são escassas as informações sobre a estabilidade da mucilagem durante o armazenamento sob refrigeração. Isso torna importante caracterizar e averiguar a estabilidade físico-química da mucilagem e a influência da estação do ano, para fins de utilização de mucilagem de palma na indústria de alimentos.

Com isso, a hipótese deste trabalho prevê que *a estação do ano, o clone e o armazenamento alteram as propriedades da mucilagem de palma dos gêneros Opuntia e Nopalea.* Este trabalho foi estruturado na forma de artigo (seguindo as normas da revista) que foi submetido ao periódico científico Food Research International, qualis A1, fator de impacto 4,972.

CHAPTER 1 – CHARACTERIZATION OF MUCILAGE FROM CLONES OF *OPUNTIA* AND *NOPALEA* PRICKLY PEAR CACTUS HARVESTED IN THE WET AND DRY SEASONS OF THE BRAZILIAN SEMIARID REGION

ABSTRACT: The season of the year and the clone can interfere with the physicochemical, biochemical and sensory properties of mucilage. This study proposes to characterize hydrated and refrigerated mucilage obtained from cladodes of clones of prickly pear cactus harvested during the wet and dry seasons in the semiarid region 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 06h00, chopped, ground and homogenized in ethanol. The extracted mucilage was oven-dried, the main bands in the infrared region were characterized, and then the mucilage was hydrated and kept at 5 °C for 12 days. Physicochemical and biochemical 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^+ contents, for the three clones. The OEM clone also exhibited significant increases in pH, Na^+ content 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^+ contents). The spectroscopic profile was similar for all studied clones. Principal component analysis allowed the formation of clusters between seasons and conservation times. In conclusion, the physicochemical and biochemical traits of mucilage vary according to the clone and the season of the year. Therefore, they should be considered for the use of mucilage in the food industry.

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

RESUMO: A estação do ano e o clone podem interferir nas propriedades físico-químicas, bioquímicas e sensoriais da mucilagem. Neste estudo, objetivou-se caracterizar a mucilagem hidratada e refrigerada obtida a partir de cladódios de clones de palma colhidos nas estações chuvosa e seca no Semiárido do Brasil. Os cladódios dos clones *Opuntia stricta* [Haw.] Haw. (Orelha de Elefante Mexicana-OEM) e *Nopalea cochenillifera* Salm Dyck (clones IPA Sertânia-IPA e Miúda-MIU) foram colhidos às 6h, cortados, triturados e homogeneizados em etanol. A mucilagem extraída foi seca em estufa, caracterizada as principais bandas na região do infravermelho e, em seguida, hidratada e mantida a 5 °C por 12 dias. Análises físico-químicas e bioquímicas foram realizadas no dia zero e aos 12 dias. Os cladódios colhidos na estação seca obtiveram maior rendimento de mucilagem, sólidos solúveis, carboidratos solúveis totais e teor de K^+ para os três clones. O clone OEM exibiu ainda acréscimos significativos do pH, teor de Na^+ e condutividade elétrica na estação seca quando comparada a estação chuvosa. Além disso, a mucilagem extraída dos cladódios de *Opuntia* não alterou os teores de carboidratos, acidez titulável e proteínas solúveis totais. Já IPA e MIU caracterizaram-se com parâmetros que se mantiveram estáveis na conservação (compostos fenólicos, acidez titulável, teores de K^+ e Na^+). O perfil espectroscópico é similar para todos os clones estudados. A análise de componentes principais revelou o agrupamento entre as estações do ano e o tempo de conservação. Conclui-se que, as características físico-químicas e bioquímicas da mucilagem variam de acordo com o clone e a estação do ano, logo devem ser consideradas para o uso da mucilagem na indústria de alimentos.

Palavras-chave: *Opuntia stricta*; *Nopalea cochenillifera*; mucilagem; infravermelho; principal component analysis (PCA).

1 INTRODUCTION

The prickly pear cactus is found in several regions of the world (e.g. Mexico, Tunisia, Brazil and Ethiopia), in 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 for the production of dyes (Ochoa & 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; Silva 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 (Galvão et al., 2018; Pereira et al., 2013). At present, prickly pear cactus biomass is considered a valuable raw material for biomolecules with applications in packaging (Gheribi & Khwaldia, 2019).

The development of new biomaterials from agricultural by-products and wastes is not only a worldwide trend, but 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 breads (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 cactus (Sáenz et al., 1998a). 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., 2004b), 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. 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 presenting crassulacean acid metabolism (CAM) an active composition changes according to hours (Rodríguez-Félix & Cantwell, 1988).

The physicochemical, biochemical and sensory 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). In view of this, a more in-depth study is warranted to elucidate the mucilage yield potential of other clones of the genera *Opuntia* and *Nopalea* as well as the effect of the season of the year on the physicochemical and biochemical composition of hydrated and refrigerated mucilage.

Therefore, the present study was conducted 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.

2 MATERIALS E METHODS

2.1 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, located 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^{-1} (Pereira et al., 2015). The soil in the growing area was 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 was 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 procedures were performed whenever necessary. No phytosanitary treatment was necessary.

The cladodes of the three clones were collected in two seasons -wet and dry- and harvested always at 06h00 (maximum time of two hours). In the wet season, the cladodes were collected throughout the month of 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.

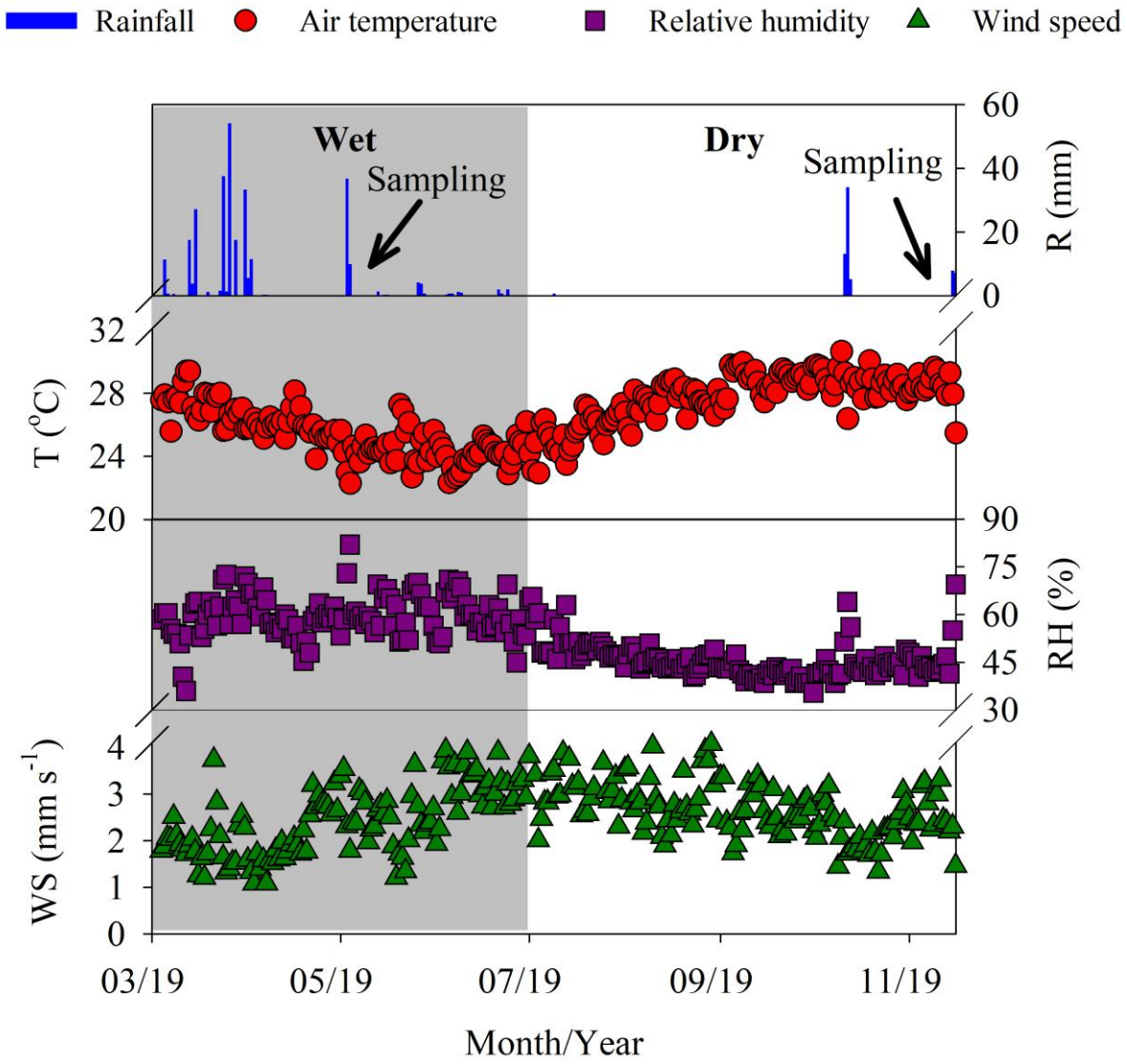

Figure 1. Rainfall (mm), air temperature (° C), relative humidity (%) and wind speed (mm s-1) between the months of March to June 2019 (wet season) and July to November 2019 (dry season), in the municipality of Serra Talhada-PE, Brazil. Source: INMET, 2020.

2.2. 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 (Philips Walita, ri7775, 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. Afterwards, 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: $MY = [(Wf/Wi)*100]$, where *MY* is the mucilage yield in percentage values (%), on a fresh-weight basis; *Wf* is the final weight of the powdered mucilage (g); and *Wi* is the initial weight of the whole cladodes (g).

2.3 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.

2.4. 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) (Moretti et al., 1998). 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.

2.5. Potential of hydrogen (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 following formula was used: $TA = [(N*V*Eq\ citric\ acid)/v]$, 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 Tillmans' solution, following the method described by IAL (2008). For this purpose, 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: $Vit.\ C = V \times F \times 100/S$, where *V* = volume of Tillmans solution used in the titration (mL); *F* = Tillmans solution correction factor; and *S* = sample volume (mL). Results were expressed in mg of ascorbic acid per 100 g DW.

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

The sodium and potassium 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}.

2.7. 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) were 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 were 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) was used as an external standard. The TSP content was expressed in mg of soluble protein per 100 g DW.

2.8 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 region of 4000-400 cm^{-1}, under 8 cm^{-1} resolution, 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 was not performed on hydrated mucilage to prevent the water from interfering with the functional groups characteristic of mucilage.

2.9 Statistical analysis

Shapiro-Wilk's test was applied for normality of residuals and Levene's test for homogeneity between variances. When these two assumptions were met, analysis of variance was used for the physicochemical and biochemical data, at the 5% significance level, by Fisher-Snedecor's F-test. In significant 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, 2019) 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).

3 RESULTS

The mucilage yields obtained from the cladodes of the IPA Sertânia (IPA), Miúda (MIU) and Orelha de Elefante Mexicana (OEM) clones were higher in the dry season than in the et season ($p < 0.05$, Table 1). Furthermore, the mucilage yield of MIU was higher than those of the two other clones (Table 1).

Table 1. Mucilage yield (%) of IPA Sertânia (IPA), Miúda (MIU) and Orelha de Elefante Mexicana (OEM) in two seasons, wet and dry.

Clones	Wet	Dry
IPA	1.5	5.0
MIL	2.4	7.3
OEM	1.1	2.3

The SS and TC contents of the mucilage were higher in the dry season, regardless of the clone (Fig. 2). In the MIU clone, the SS content of the mucilage remained stable between days 0 and 12 ($p > 0.05$, Fig. 2B). The same was observed for the TC content of the mucilage from the IPA and OEM clones (Fig. 2E and 2F).

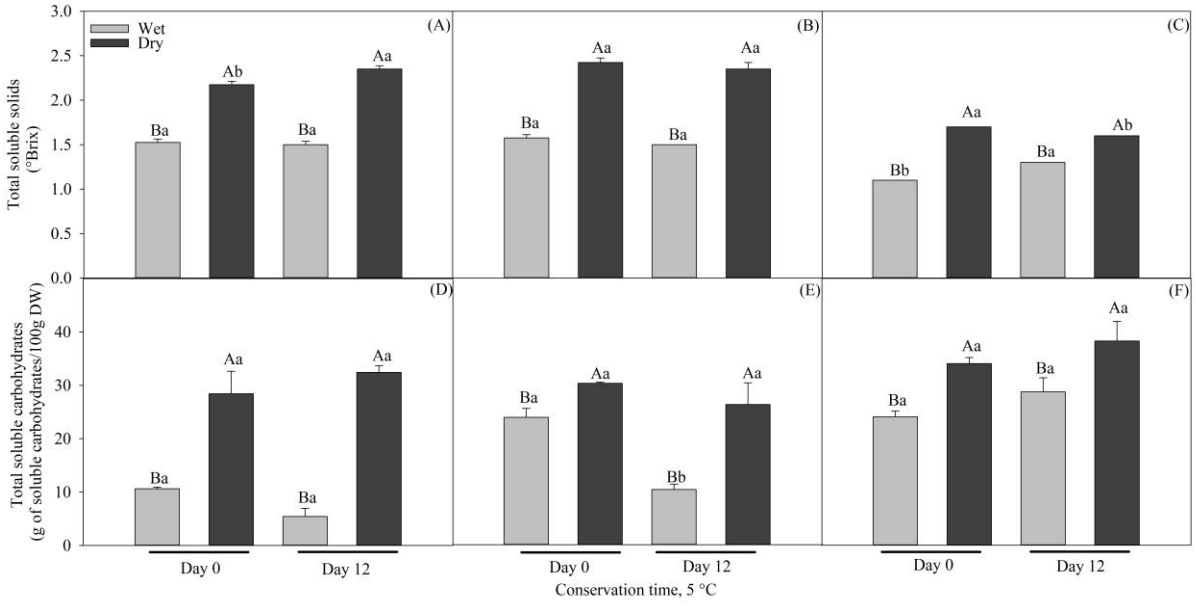

Fig. 2. Content of soluble solids and total carbohydrates in mucilage extracted from prickly pear cactus cladodes, IPA, Miúda and Orelha de Elefante Mexicana collected in the wet season and in the dry season and stored for 12 days at 5 °C. Clones: IPA Sertânia (A and D), Miúda (B and E) and Orelha de Elefante Mexicana (C and F). The bars represent the standard deviation of the mean. The letters represent a statistical difference between the averages by the Tukey test, at 5% probability, the uppercase letters for the season and lowercase letters for the conservation days.

On day 0, the pH of mucilage extracted from the OEM clone was higher in the dry season than in the Wet season (Fig. 3C). This was not true for the other clones (Fig. 3A and B). The three clones showed similar TA and vit. C contents on day 0, regardless of whether they were harvested in the dry or wet season (Fig. 3 D, E, F, G, H and I).

During storage, the pH of mucilage from all clones increased (Fig. 3A, B and C), whereas TA remained similar (from 0 to 12 days) (Fig. 3D, E and F). Vitamin C levels decreased throughout the storage days in the mucilage produced from the cladodes of the IPA and OEM clones (Fig. 3G and I). From the start to the end of preservation, the content of this phytochemical in the mucilage of the MIU clone did not change (Fig. 3H).

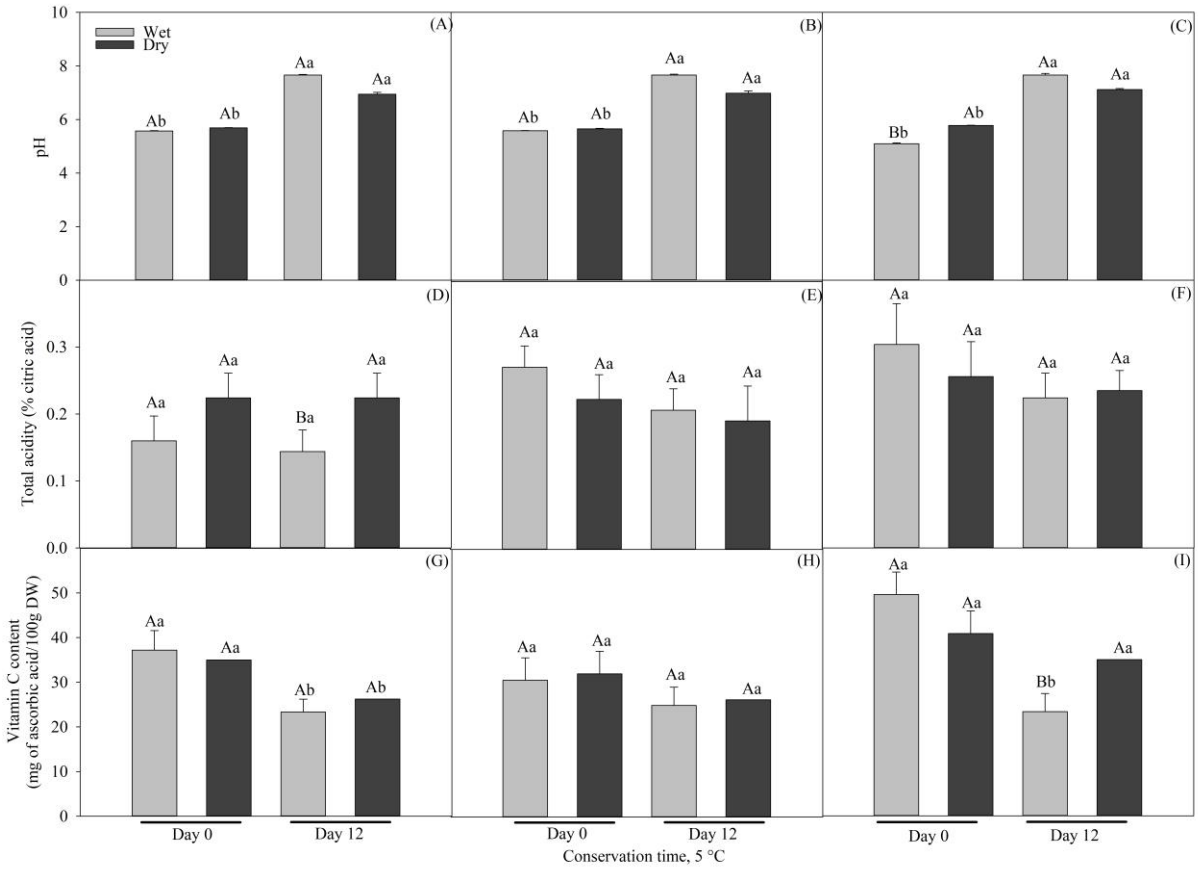

Fig. 3. pH, total acidity and vitamin C content in mucilage extracted from prickly pear cactus cladodes, IPA, Miúda and Orelha de Elefante Mexicana collected in the wet season and in the dry season and stored for 12 days at 5 ° C. Clones: IPA Sertânia (A; D and G), Miúda (B; E and H) and Orelha de Elefante Mexicana (C; F and I). The bars represent the standard deviation of the mean. The letters represent statistical difference between the averages by the Tukey test, at 5% probability, the capital letters for the time of the year and the lower letters for the conservation days.

On day 0, the K^+ content of the mucilage was higher in the dry season, in all clones ($p<0.05$, Fig. 4A, B and C), whereas the Na^+ content increased only in the OEM clone (Fig. 4F). Electrical conductivity, in turn, was higher in the mucilage from the IPA and OEM clones in the dry season (Fig. 4G and I).

Storage provided stability in the K^+ and Na^+ contents of the mucilage produced from the cladodes of the IPA and MIU clones (Fig. 4B, C, E and F). EC in the mucilage extracted from the MIU clone remained constant throughout the storage period (Fig. 4H).

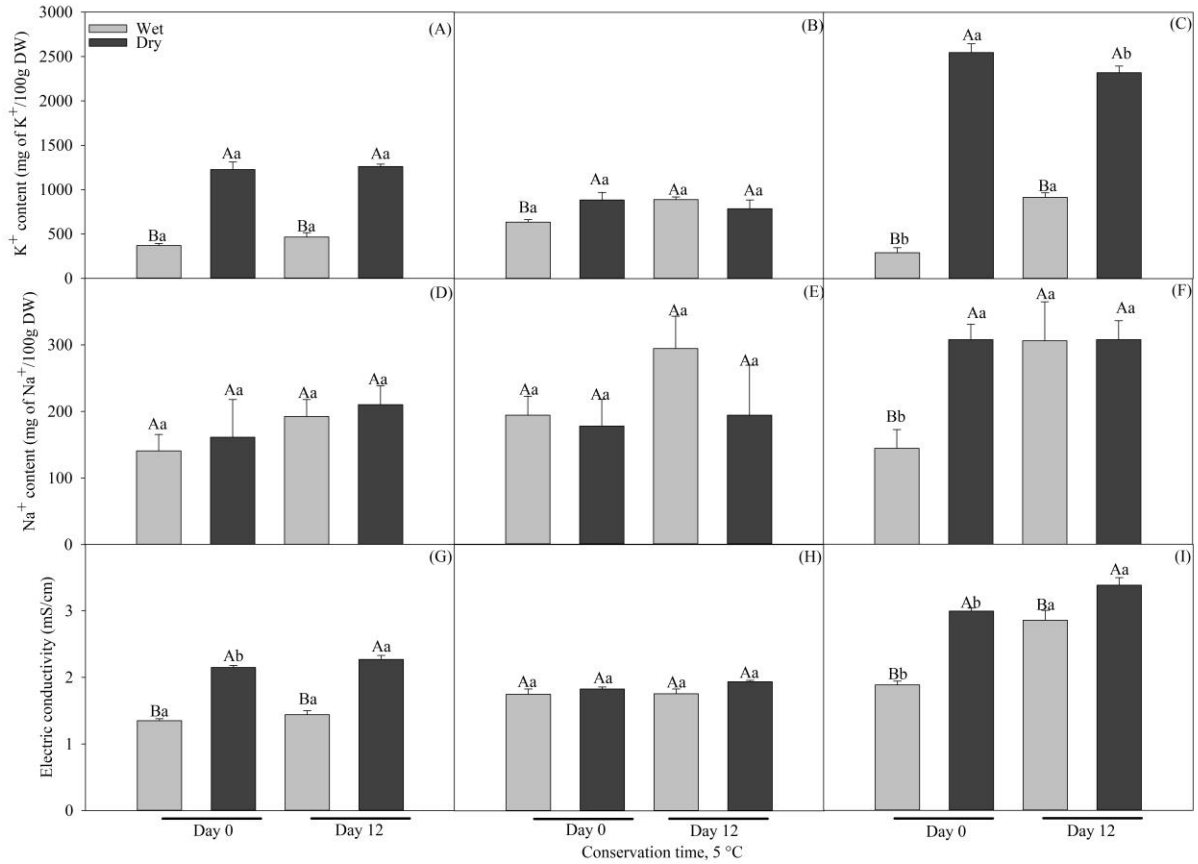

Fig. 4. K^+, Na^+ and electrical conductivity in mucilage extracted from prickly pear cactus cladodes, IPA, Miúda and Orelha de Elefante Mexicana clones harvested in the rainy and dry

season 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). The bars represent the standard deviation of the mean. The letters represent statistical difference between the averages by the Tukey test, at 5% probability, the capital letters for the time of the year and the lower letters for the conservation days.

The mucilage obtained from cladodes of prickly pear cactus harvested in the dry season showed lower TPC levels as compared with those harvested the wet season, in the IPA and MIU clones (Fig. 5A and B). This result is similar to that observed for the mucilage of MIU clone, whose TSP content also was also lower in the dry season (Fig. 5E).

During storage, only the mucilage extracted from the OEM clone, in both seasons, exhibited higher levels of TPC ($p < 0.05$, Fig. 5C). As for the TSP content, a significant reduction of this metabolite was observed in the IPA clone in both seasons and conservation times (Fig. 5D).

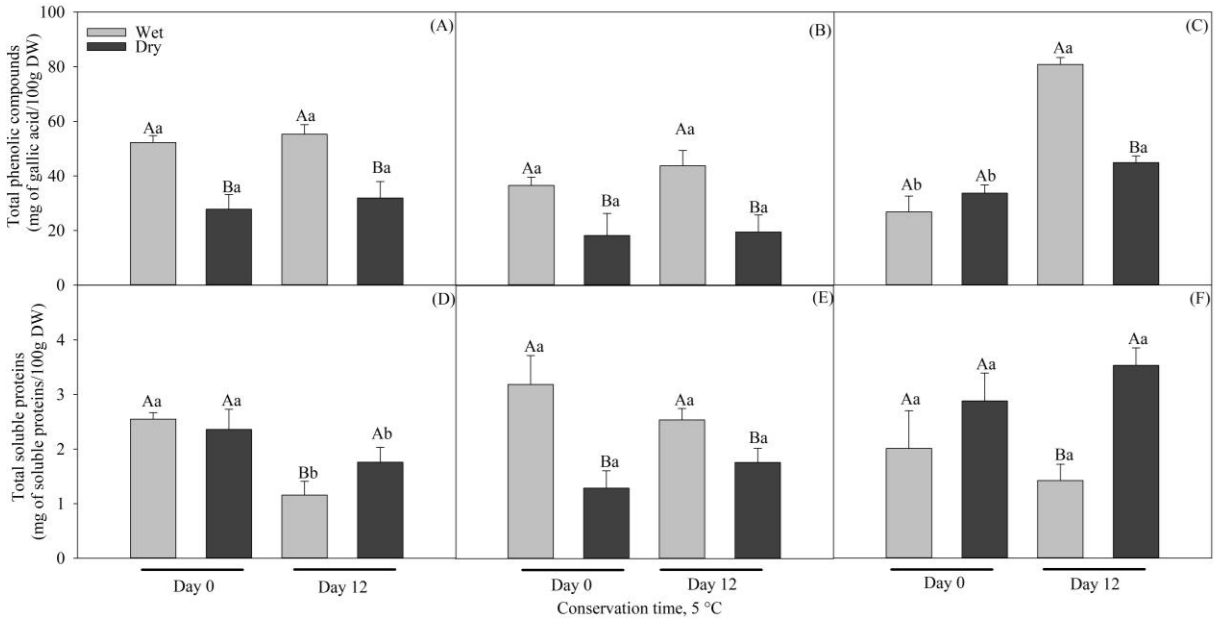

Fig. 5. Total phenolic compounds and total soluble proteins in mucilage extracted from prickly pear cactus cladodes, IPA, Miúda and Orelha de Elefante Mexicana clones harvested in the rainy season in the dry season and stored for 12 days at 5 °C. Clones: IPA Sertânia (A, D and G), Miúda (B, E and H) and Orelha de Elefante Mexicana (C, F and I). The bars represent the standard deviation of the mean. The letters represent statistical difference between the averages by the Tukey test, at 5% probability, the capital letters for the time of the year and the lower letters for the conservation days.

The general profile of the spectra in the infrared region of the mucilage from the IPA, MIU and OEM clones harvested in different seasons (wet and dry) was described (Fig. 6). The main bands were observed at 3331 cm^{-1}, 2926 cm^{-1}, 1734 cm^{-1}, 1620 cm^{-1}, 1347 cm^{-1} and a more intense one at 1044 cm^{-1} (Fig. 6).

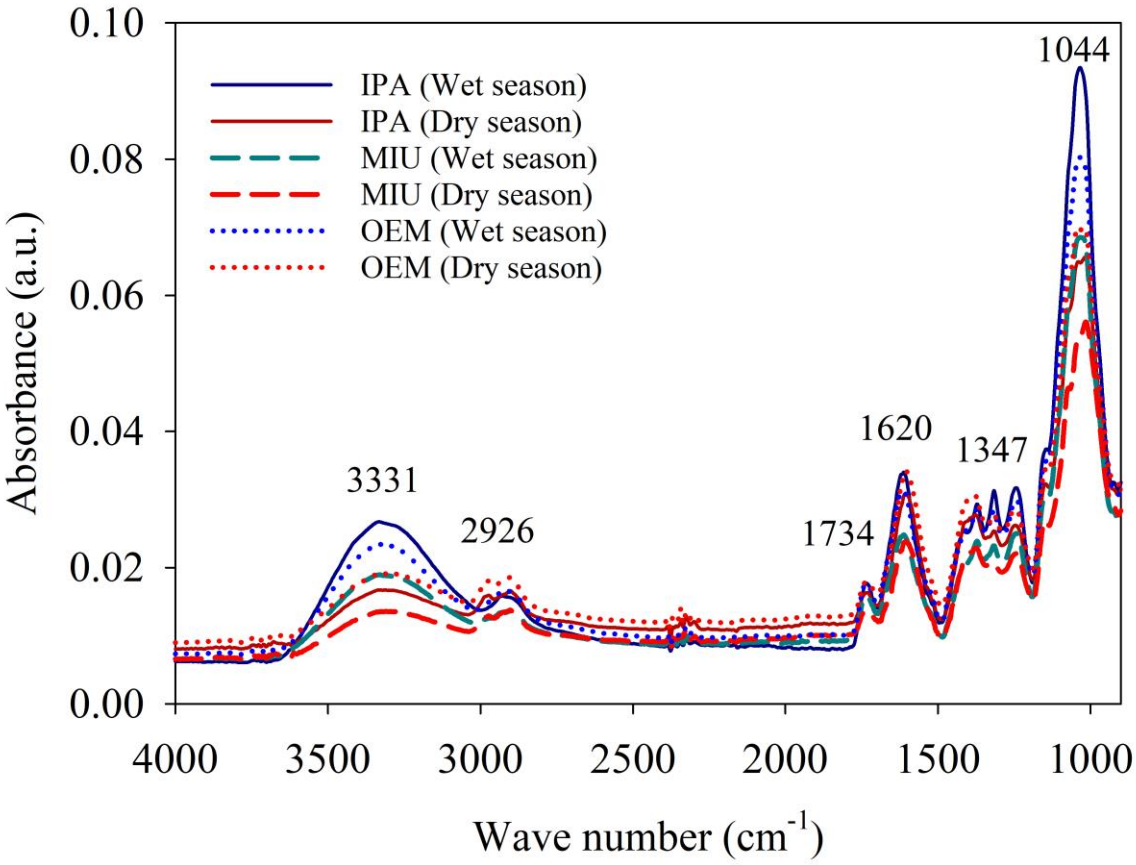

Fig. 6. Medium spectra of powdered mucilage FTIR extracted from cladodes of the IPA (A), Miúda (B) and Orelha de Elefante Mexicana (C) clones harvested in the wet and dry seasons.

According to principal component analysis (PCA), the three principal components explain 91% of the total variation of the data (PC1 = 39%, PC2 = 31% and PC3 = 21%) (Fig. 7). The most evident group formation was based on the season, with the dry season located more to the right in Fig. 7B and the wet season to the left in Fig. 7B. However, the Dry-OEM-0 set in PC1 was positively associated with pH, Na^+, K^+, EC and TC (Fig. 7A). In PC2, the Wet-IPA-0 set was positively correlated with TPC and FTIR and negatively correlated with vit. C and TA, whereas the opposite occurred with the Wet-OEM-0 set. The positive association of FTIR with TPC and its negative association with vit. C and TA are noteworthy.

In PC3, variations in the Wet-MIU-0, Dry-MIU-0 and Dry-IPA-0 sets were explained to a greater extent by the TSP and SS variables.

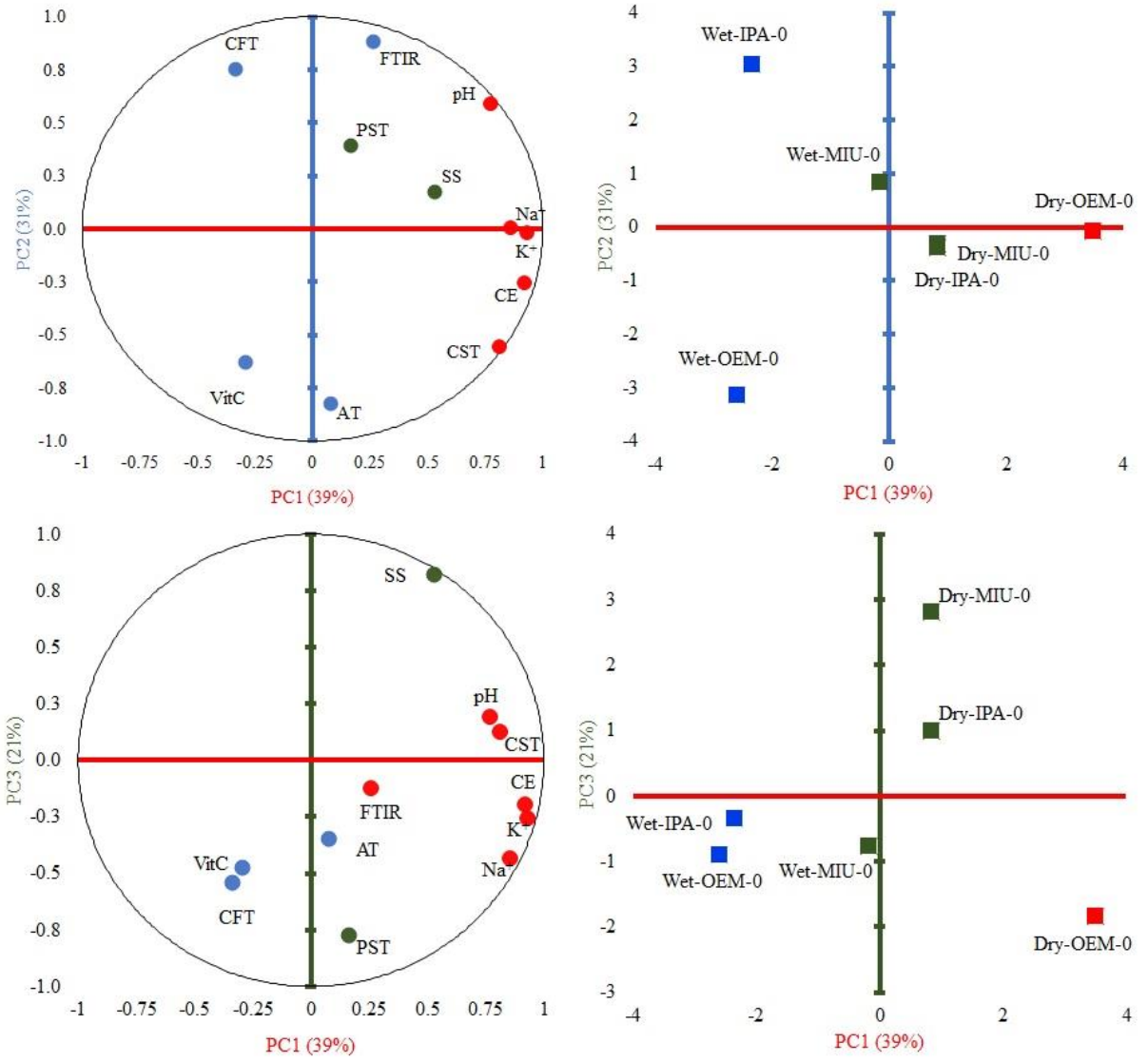

Fig. 7. Scores, obtained by the PCA from physico-chemical and biochemical data of powdered mucilage extracted from cladodes of the IPA Sertânia, Miúda and Orelha de Elefante Mexicana clones harvested in the wet and dry seasons.

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. 8). 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 a 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.

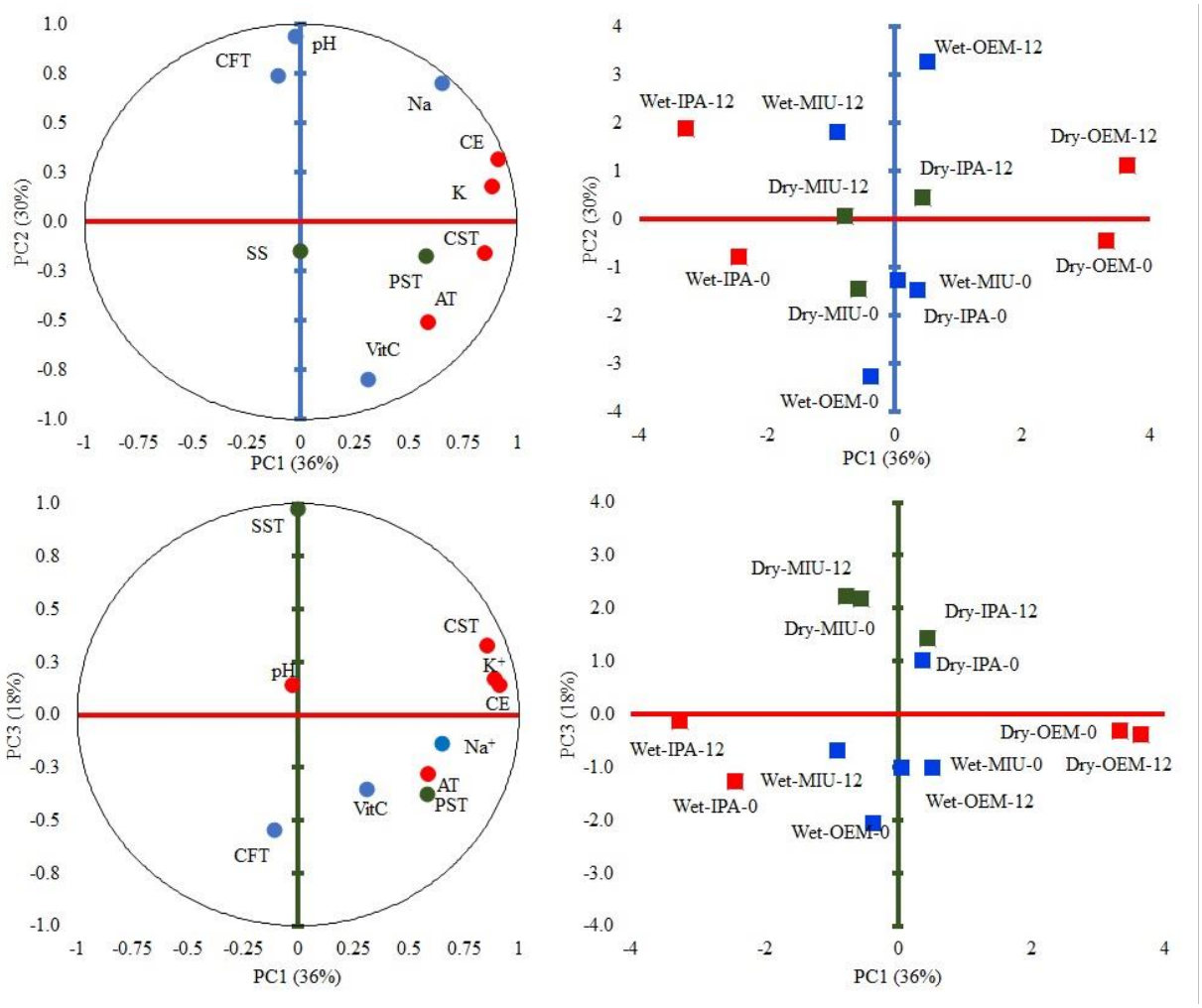

Fig. 8. Scores, obtained by the PCA from physico-chemical and biochemical data of powdered mucilage extracted from cladodes of the IPA Sertânia, Miúda and Orelha de Elefante Mexicana clones harvested in the wet and dry seasons and stored for 12 days.

4 DISCUSSION

In this study, we examined some important physicochemical and biochemical properties in the characterization of mucilage for the purposes of 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 wet season had a total accumulated precipitation of 285 mm, average air temperature of 25 °C and 59% relative humidity (Fig. 1). In the dry season, total accumulated precipitation was 68 mm, average air temperature was 28 °C and relative humidity was 43% (Fig. 1). These climatic differences resulted in significant differences in mucilage yield (Tab. 1). 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 and biochemical parameters of the mucilage, as indicated in the PCAs (Fig. 7 and 8). The mucilage obtained from cladodes harvested in the dry season exhibited significantly higher SS, TC (Fig. 2) and K^+ values in all studied clones (Fig. 4). The mucilage extracted from the OEM clone during the dry season also showed higher pH (Fig. 3C), Na^+ (Fig. 4F) and EC values (Fig. 4I). A higher TC content in the dry season was also reported by Ribeiro et al. (2010) and by Falcão et al. (2013) 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.

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 greater 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, as shown in Table 1.

The mucilage extracted from the IPA cladodes and kept at 5 ºC for 12 days maintained its TC (Fig. 2), K^+, Na^+ (Fig. 4) and TPC (Fig. 5A and B) levels in relation to the start of the experiment. These results are good indicators for the formulation of an edible coating, since fruits or vegetables require refrigeration. Conversely, for the mucilage extracted from the OEM clone during the wet season, storage increased the levels of SS, K^+, Na^+ and CE (Fig. 4C and I). 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.

9). 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 et al. (2010), 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 were lower than those reported by Medina et al. (2010), 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. 2010).

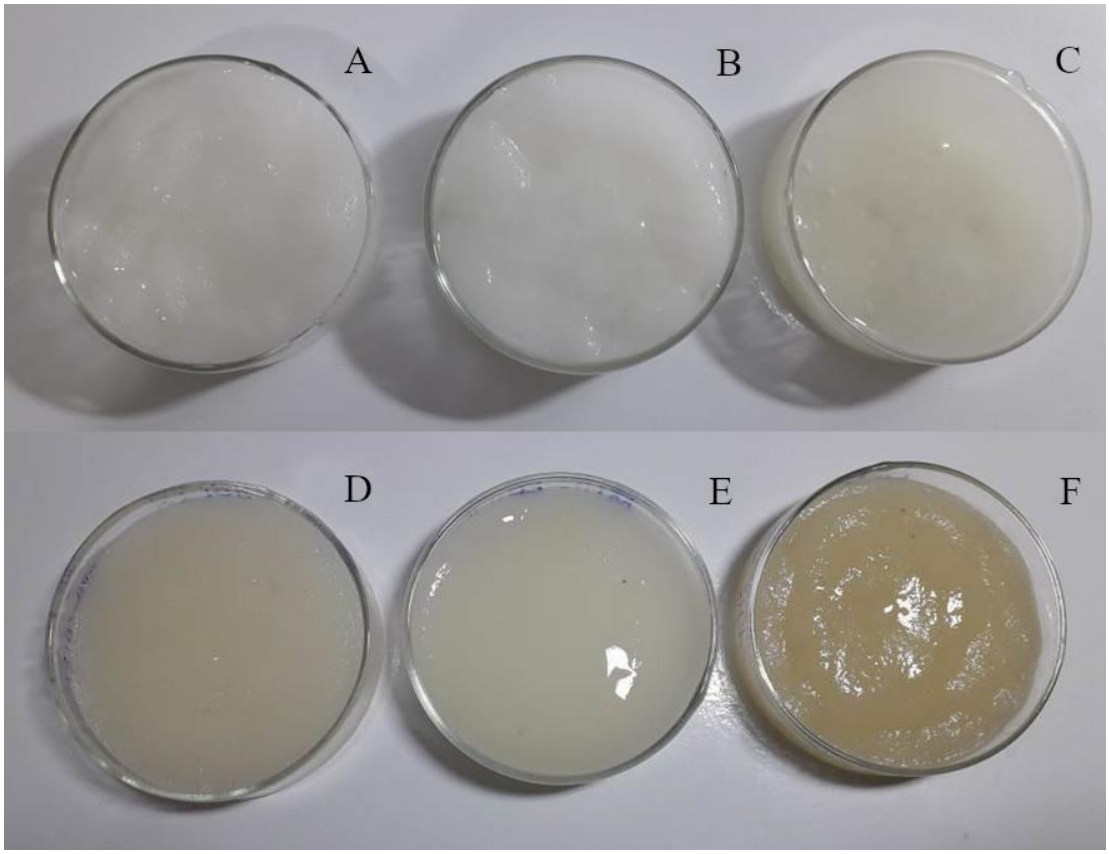

Fig. 9. Photographs of prickly pear cactus mucilages extracted from IPA (A and D), Miúda (B and E) and Orelha de Elefante Mexicana (C and F) at the beginning (A, B and C) and at 12 days (D, E and F) under refrigeration, harvested in the wet season.

Mucilage is composed of a complex mixture of macromolecules with a larger portion of polysaccharides (~14% of the dry weight) (Goldstein et al., 1991). 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). 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 (Fig. 6) (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).

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 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).

Principal component analysis allowed the study of the relationships between the physicochemical and biochemical data for each group of samples (clones, seasons and conservation times) (Figures 7 and 8). Groups were formed for the dry and wet seasons (Fig. 7) and there was a trend for the formation of groups between the treatments of 0 and 12 days (Fig. 8), for all analyzed clones. These differences in the clusters formed between the studied cactus genera according to harvest time and storage period reinforce the changes in the physicochemical and biochemical 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.

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. Finally, the data reveal the importance of systematically handling the raw material, especially with regard to the environmental conditions and the cactus clones to be used for industrial purposes.

5 CONCLUSIONS

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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7 ANNEXES

7.3 Highlights

1 – Mucilage production can be enhanced by the time of year and clone in the Semi-Arid;

2 – The Nopalea genus has a strong potencial for use in the food industry;

3 – Harvesting in dry periods resulted in higher mucilage yield;

4 – The physicochemical stability of the mucilage was more evident for the genus Nopalea.

7.4 Graphic abstract