

# Influence of irrigation, cladode size, harvest time and addition of citric acid on the properties of cactus mucilage<sup>1</sup>

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## ABSTRACT

Cactus is a highly resistant crop, and offers benefits in its mucilage for the industry due to its physicochemical properties. This study aimed to analyze the effect of irrigation managements, harvest time, cladode size and citric acid addition during the processing on the agro-industrial yield and physicochemical properties of mucilage extracted from cladodes of *Nopalea cochenillifera* Miúda clone cultivated under rainfed and irrigated conditions. The yield, soluble solids content, titratable acidity, pH, K<sup>+</sup> and Na<sup>+</sup> contents, and the electrical conductivity were measured in the mucilage. The management practices in the field resulted in changes in the studied phytochemicals. For the rainfed cultivation, the night harvest of the biggest cladodes increased the K<sup>+</sup> and soluble solids contents. The addition of citric acid to the mucilage extraction resulted in higher amounts of soluble solids, titratable acidity and electrical conductivity and lower pH values. The highest water availability promoted higher mucilage yields.

**KEYWORDS:** *Nopalea cochenillifera* (L.) Salm-Dyck, Cactaceae, hydrocolloid, titratable acidity.

## RESUMO

Influência da irrigação, tamanho do cladódio, época de colheita e adição de ácido cítrico nas propriedades da mucilagem de palma forrageira

A palma forrageira é uma cultura altamente resistente, e oferece benefícios em sua mucilagem para a indústria devido às suas propriedades físico-químicas. Objetivou-se analisar o efeito do manejo de irrigação, época de colheita, tamanho do cladódio e adição de ácido cítrico durante o processamento no rendimento agroindustrial e nas propriedades físico-químicas de mucilagem extraída de cladódios de *Nopalea cochenillifera* clone Miúda cultivados em sequeiro e irrigados. Foram medidos o rendimento, teor de sólidos solúveis, acidez titulável, pH, teor de K<sup>+</sup> e Na<sup>+</sup>, e condutividade elétrica na mucilagem. As práticas de manejo no campo resultaram em alterações nos fitoquímicos estudados. No cultivo de sequeiro, a colheita noturna dos cladódios maiores aumentou os teores de K<sup>+</sup> e sólidos solúveis. A adição de ácido cítrico à extração da mucilagem resultou em maiores quantidades de sólidos solúveis, acidez titulável e condutividade elétrica e menores valores de pH. A maior disponibilidade hídrica promoveu maiores rendimentos de mucilagem.

**PALAVRAS-CHAVE:** *Nopalea cochenillifera* (L.) Salm-Dyck, Cactaceae, hidrocoloide, acidez titulável.

## INTRODUCTION

Cacti are grown mainly for fruit consumption, forage and dye production (Ochoa & Barberab 2017, Jardim et al. 2020a, Jardim et al. 2021). Currently, the cactus biomass is considered a valuable raw material for value-added biomolecules used in various industrial applications, such as in the pharmaceutical

industry (Trombetta et al. 2006); flocculating agents for treating contaminated water, e.g., with heavy metals (Nharingo & Moyo 2016, Vecino et al. 2016); civil construction (Ventolà et al. 2011); and also in the food industry, with application in edible coatings (Morais et al. 2019). Another alternative for its use is the making of food packaging film (Gheribi & Khwaldia 2019).

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The *Nopalea cochenillifera* (L.) Salm-Dyck mucilage is considered a hydrocolloid rich in non-starch polysaccharides (Nava et al. 2018), with a high molecular weight (Matsuhiro et al. 2006). Its main composition consists of sugars such as arabinose, galactose and xylose (Trachtenberg & Mayer 1981, Liguori et al. 2020). It has filmogenic, highly elastic and biodegradable properties, is atoxic and has a slightly acidic pH (Prajapati et al. 2013), being considered a sustainable alternative for the production of food packaging to replace plastic packaging derived from oil (Gheribi et al. 2018). It is also known that cladodes have nutrients such as vitamins, minerals and functional compounds such as phenolic antioxidants (Stintzing & Carle 2005) with high antioxidant capacity (Nabil et al. 2019), which make them candidates for use in food-related products.

Several studies have shown the importance of agronomic practices in the production of cactus biomass and the use of cladodes in the industry (Trombetta et al. 2006, Jardim et al. 2021, Saraiva et al. 2021, Alves et al. 2022). In addition, the use of fertilization levels (Saraiva et al. 2021), irrigation (Lima et al. 2018), crop orientation and cropping systems (Jardim et al. 2021, Saraiva et al. 2021, Alves et al. 2022) are practices that favor the increase in the number of cladodes and biomass yield. On the other hand, as far as we know, it is still unknown to what extent crop practices may influence the characteristics of cactus mucilage.

The physicochemical properties of mucilage may change depending on the species of the *Opuntia* genus (Rodríguez-González et al. 2014) and according to the plant age and season (Ribeiro et al. 2010), as well as different weather conditions (Du Toit et al. 2020). Given this information, it is extremely important to monitor its cultivation conditions, in relation to the mucilage physicochemical properties. In addition, in studies carried out worldwide, the genus studied has been *Opuntia*. So, more information is needed about the *Nopalea* genus for food purposes, since it is also a genus with significant relevance and high nutritional and industrial values. Therefore, this study aimed to analyze the effect of irrigation managements, harvest time, cladode size and citric acid addition during the processing on the agro-industrial yield and physicochemical properties of mucilage extracted from cladodes of the *Nopalea* genus.

## MATERIAL AND METHODS

Fresh cladodes of prickly pear cactus [*Nopalea cochenillifera* (L.) Salm-Dyck] Miúda clone were harvested in two experimental areas located at the Universidade Federal Rural de Pernambuco, in Serra Talhada, Pernambuco State, Brazil (7°59'S, 38°15'W and 431 m of altitude), being one rainfed and the other irrigated. The climate of this region is classified as BSh (semi-arid hot), with rainy summer and dry winter. The rainfed area was planted in January 2014, with spacing of 1.0 m between rows and 0.4 m between plants (25,000 plants ha<sup>-1</sup>). Before cultivation, an initial soil preparation was carried out, consisting of plowing, harrowing and furrowing, and the cladodes were inserted in the soil at 50 % of their lower base.

The cultivation under irrigation started on January 20 (2016), with spacing of 1.0 m between rows and 0.20 m between plants (50,000 plants ha<sup>-1</sup>), and initial soil preparation carried out similarly to the rainfed experiment. The irrigation management of the area was carried out using drip tapes (1.75 L h<sup>-1</sup>; 100 kPa), based on the crop evapotranspiration (Lima et al. 2018). For both experimental areas, two chemical fertilizations were carried out, the first in January 2016 and the second in January 2017, with the formulation 14-00-18 + 16 S, using 525 kg ha<sup>-1</sup>, i.e., 73.5 kg ha<sup>-1</sup> of N, 94.5 kg ha<sup>-1</sup> of K<sub>2</sub>O and 84 kg ha<sup>-1</sup> of S, and crop treatments were carried out when necessary for the plants to maintain a full development. The soil in the experimental areas was classified as Cambissolo (Jardim et al. 2020b), equivalent to Inceptisol (USDA 2014) (Table 1).

The harvest of the cladodes grown under rainfed condition was carried out in November and December 2017, while those from irrigated cultivation were harvested in January 2018. For their harvest, the collection was standardized at third order cladode plants and the materials from both areas were present in the vegetative phase. The weather conditions during the harvest period were monitored from an automatic weather station (located at 60 m from the experiment area) for air temperature, relative humidity, wind speed and rainfall (Figure 1).

Cladodes with lengths between 100 and 230 mm were harvested at two times during the day: 5 a.m. and 8 p.m. (Panta-Araújo et al. 2021). In other assay, the cladodes were harvested and classified into two sizes: 100-230 mm in length (called medium size - M)

Table 1. Soil chemical and physical characteristics.

| Soil physical properties (0-0.20 m layer) |     |        |     |                            |                  |                            |                 |                            |      |      |      |
|---|-----|--------|-----|----------------------------|------------------|----------------------------|-----------------|----------------------------|------|------|------|
| BD (g cm <sup>-3</sup> )                  |     | φt (%) |     | Sand (g kg <sup>-1</sup> ) |                  | Silt (g kg <sup>-1</sup> ) |                 | Clay (g kg <sup>-1</sup> ) |      |      |      |
| 1.5                                       |     | 42.3   |     | 828.6                      |                  | 148.3                      |                 | 23.2                       |      |      |      |
| Soil chemical properties (0-0.20 m layer) |     |        |     |                            |                  |                            |                 |                            |      |      |      |
| pH  | ECe | P      | OC  | OM                         | Ca <sup>2+</sup> | K <sup>+</sup>             | Na <sup>+</sup> | Mg <sup>2+</sup>           | SB   | CEC  | V    |
| 6.0                                       | 0.3 | 169    | 4.6 | 7.9                        | 3.5              | 13.8                       | 1.1             | 1.9                        | 20.3 | 20.9 | 97.2 |

BD: bulk density; φt: total porosity; ECe: electrical conductivity of the saturated soil extract (dS m<sup>-1</sup>); P: phosphorus (mg dm<sup>-3</sup>); OC: organic carbon (g kg<sup>-1</sup>); OM: organic matter (g kg<sup>-1</sup>); Ca<sup>2+</sup>: calcium (cmol<sub>c</sub> dm<sup>-3</sup>); K<sup>+</sup>: potassium (cmol<sub>c</sub> dm<sup>-3</sup>); Na<sup>+</sup>: sodium (cmol<sub>c</sub> dm<sup>-3</sup>); Mg<sup>2+</sup>: magnesium (cmol<sub>c</sub> dm<sup>-3</sup>); SB: sum of bases (cmol<sub>c</sub> dm<sup>-3</sup>); CEC: cation exchange capacity (cmol<sub>c</sub> dm<sup>-3</sup>); V: base saturation (%).

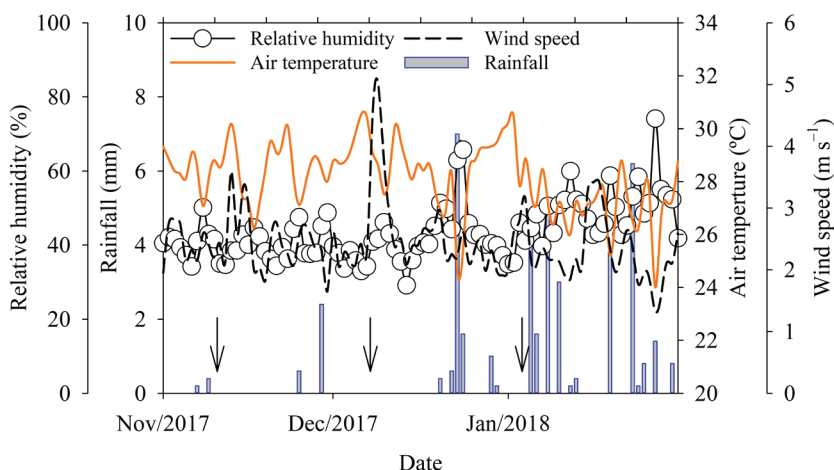


Figure 1. Weather conditions during the harvest of prickly pear cactus cladodes [*Nopalea cochenillifera* (L.) Salm-Dyck] Miúda clone, from November 2017 to January 2018, in Serra Talhada, Pernambuco State, Brazil. Note: the arrows indicate the harvest time [i.e., November and December - rainfed harvest (non-irrigated); January - irrigated harvest].

and 231-300 mm in length (called large size - L). In another assay, the cladodes with length between 100 and 230 mm were collected at 5 a.m., then washed and processed. The mucilage was extracted by two methods: immersion in pure water (control) and in a solution containing 5 % of citric acid. In both the treatments, the parenchymal tissue was immersed for 30 min in the extraction solution. Then, the obtained mucilage was stored at 5 ± 2 °C in a commercial refrigerator (Metalfrío, mod. VB40W, São Paulo, Brazil).

For all the aforementioned tests, the mucilage was obtained as it follows: after harvested from the experimental areas, fresh *N. cochenillifera* cactus cladodes were weighed using a semi-analytical scale, washed in running water, had the extremities and the glochids/spines removed and were cut (i.e., keeping only the hydrenchyma in cube shapes). Then, the cubes were weighed and immersed in ultrapure water at 30 °C, for 30 min, and drained for 10 min, thus

obtaining the hydrated mucilage. After extraction, 20 mL aliquots of mucilage were placed in Petri dishes (9 cm in diameter), wrapped with polyvinyl chloride (PVC) plastic film with thickness of 8 μm and kept at 5 ± 2 °C in a commercial refrigerator (Metalfrío, mod. VB40W, São Paulo, Brazil) for 12 days, at a relative humidity of 85 %. Periodic analyses were performed every two days throughout the storage period.

The mucilage yield was quantified according to Sepúlveda et al. (2007). Through refractometry, the soluble solids (SS) contents were determined, and later the titratable acidity (TA) (Pregnoletto & Pregnoletto 1985). After filtering the mucilage, the content of ions, i.e., potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>), was evaluated by flame photometry (Micronal, B462, São Paulo, Brazil), and the pH and electrical conductivity (EC) were also determined (Panta-Araújo et al. 2021).

The study was carried out in a completely randomized design, using a 2 × 7 factorial scheme,

under combinations of time (seven days of evaluation) and the following factors: cultivation (rainfed and irrigated), harvest time (5 a.m. and 8 p.m.), cladodes size (100-230 mm and 231-300 mm), type of extraction (water and citric acid). Each  $2 \times 7$  factorial was analyzed separately, with three replications for each combination.

The mucilage yield, SS, pH, EC,  $K^+$  and TA were subjected to the principal component analysis (PCA) from standardized data for mean zero and standard deviation one. A PCA was performed to study the effect of each experimental factor separately, in order to obtain the maximum amount of information extracted from the data, as well as a general PCA from the averages, to study the influence of variables and the correlation between the measured mucilage attributes. The choice of the principal components was made based on the methodology proposed by Kaiser (1960), excluding eigenvalues ( $\lambda$ ) lower than 1.0. All the statistical analyses were performed using the R software.

## RESULTS AND DISCUSSION

A general principal component analysis was performed for all the studied variables (Figure 2A). Most (96.6 %) of the variance contained in the complete set of variables was retained in the first two principal components, being the first (PC1) with 57.7 %, with greater contributions from the variables SS (with a load of 0.99), TA (0.96), EC (0.82) and negatively correlated with pH, with a load of -0.90. Lower pH values were observed in the mucilage samples with citric acid and higher values of TA, SS and EC. The second principal component (PC2) was responsible for 38.9 % of the data variance, mainly due to the yield of the variables (-0.99) and the  $K^+$  (0.97), which are negatively correlated.

The PCA identification of the differences in the physicochemical composition of the mucilage was submitted to different ways of managing the cactus irrigation (Figure 2B). The variables that are farthest from the horizontal are those that most differentiate the management. Cacti grown under rainfed systems have, mainly, higher values of SS,  $K^+$ , EC and pH (Figure 2B). In addition, it was observed that the water availability promoted higher mucilage yields and, despite the occurrence of rain events before the material was harvested, under rainfed conditions the yields were lower (Figures 1 and 2). It is noteworthy

that the obtained yield was expressed as volume (mL), as a result of the dilution of mucilage in water in the extraction process, unlike other studies in which the extraction was carried out with organic solvents and the yield quantified by powder mass (g) (Gheribi et al. 2018). Thus, it was observed that the yield may vary depending on the conditions of the cladodes, as well as the mucilage extraction method. The water content influences the cell turgor, but does not necessarily increase the mucilage yield (De Wit et al. 2019, Du Toit et al. 2020). Variations in the cactus mucilage yield have also been reported by Manhivi et al. (2018) and Messina et al. (2021).

The first two principal components (PC1 and PC2) were responsible for 51.7 and 23.6 % of the total variation, respectively (Figure 2B). The SS (0.82),  $K^+$  (0.81), EC (0.92) and pH (0.56) presented loads greater than 0.5 in the PC1, with higher values under the rainfed condition. When it comes to mucilage, these constituent characteristics are extremely relevant, since they are heteropolysaccharides rich in carbohydrates (Dick et al. 2019).

The EC of the obtained mucilage had a direct influence on the composition of the PC1, showing variations between rainfed and irrigated cultivation. Variations in conductivity may be attributed to the presence of a greater number of divalent and monovalent ions, which increase conductivity (Monrroy et al. 2017). For the present study, only the monovalent ions represented by  $K^+$  and  $Na^+$  were quantified. In mucilage, the presence of electrolytes is important for the formulation of suspensions (Monrroy et al. 2017), since the electrical conductivity directly influences viscosity. 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), being not ideal for the use of this mucilage in the formulation of films and coatings, due to the decreased adhesion capacity of the coating to the product surface (Assis & Britto 2014). Based on this, the production of films and coatings would be more suitable from cladodes grown under irrigation, as they had lower EC. However, studies should be performed to confirm this hypothesis.

The TA had a greater load (0.84) in the PC2 (23.6 %). An important factor when it comes to TA is observed in relation to the viscosity of the mucilage, with a high acidity value, which may interfere with the viscosity and consequently influence some

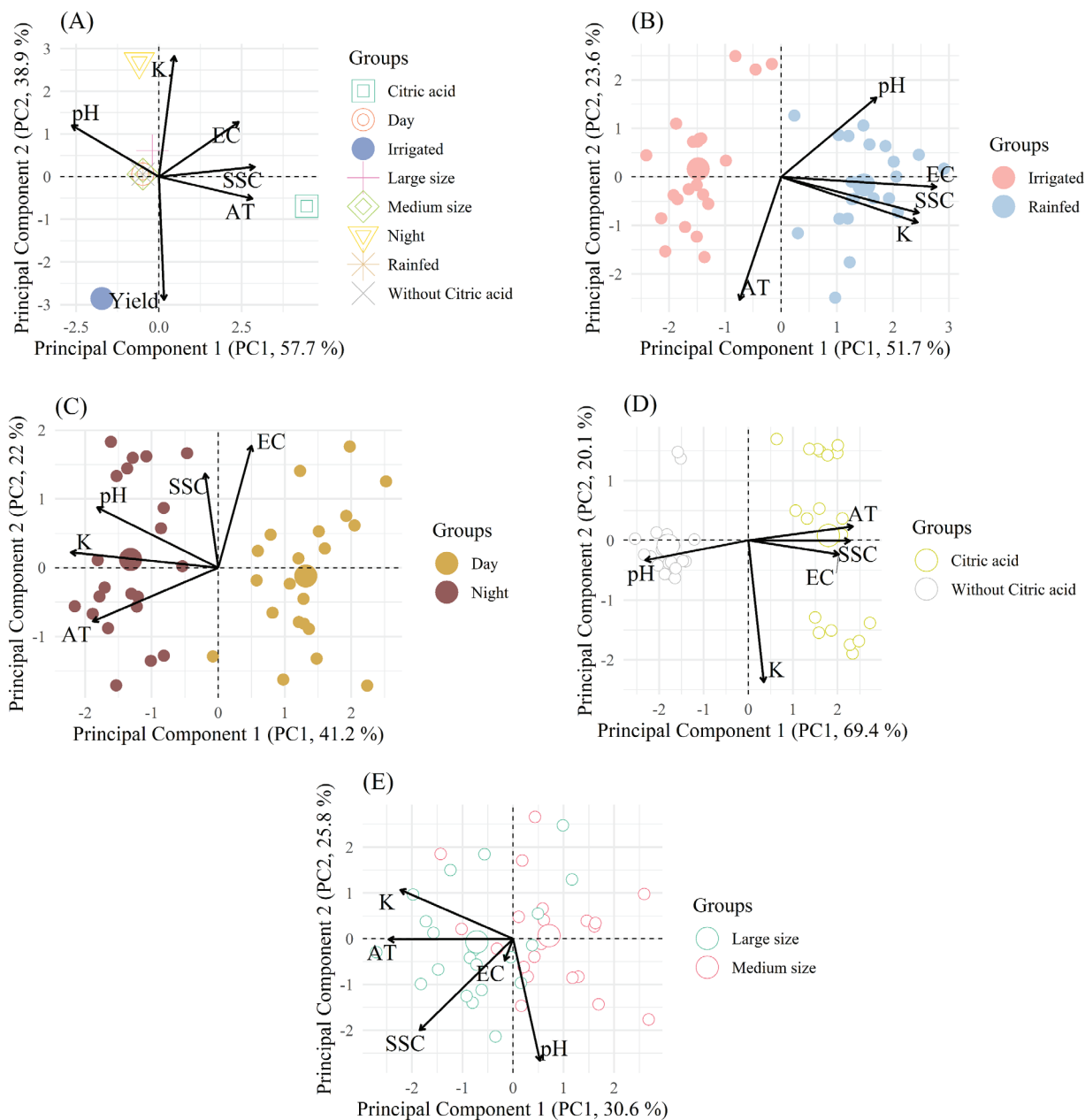


Figure 2. Biplots of the principal component analysis based on standardized averages of mucilage variables for all levels of the factors citric acid, harvest time, irrigation management and cladode size (A), under irrigated and rainfed conditions (B), under harvest time at 5 a.m. and 8 p.m. (day and night, respectively; C), with and without the addition of citric acid (D), and cladodes size (i.e., large size: L = 231-300 mm; and medium size: M = 100-230 mm) (E) from the prickly pear cactus cladodes [*Nopalea cochenillifera* (L.) Salm-Dyck] Miúda clone. Note: EC: electrical conductivity; TA: titratable acidity; SS: soluble solids contents.

applications, for example, preparation of edible coating and/or biofilm. Therefore, the increase in the values of SS, EC and K<sup>+</sup> may have contributed to a higher viscosity of the mucilage cultivated under rainfed conditions, when compared to irrigated ones (personal observations). It is believed that the dehydration of the hydnrenchyma cells (i.e.,

water-storage parenchyma) caused an increase in the concentration of solutes, resulting in a visual perception of a more viscous mucilage. According to Goldstein et al. (1991), cell dehydration may reach 82 % without irreversible damage.

The characteristics of the mucilage extracted from the cladodes harvested in the morning and

evening hours showed very different physicochemical compositions (Figure 2C). The PC1 explained 41.2 % of the variance in the data, with the highest loads for the variables  $K^+$ , TA and pH (0.91, 0.77 and 0.75, respectively). These variables showed higher values at night. The PC2 explained 22 % of the data variance, with greater importance for SS and EC, with loads of 0.56 and 0.73, respectively.

It was observed that the night harvest resulted in greater increments of important solutes such as  $K^+$  and organic acids (represented by TA). For the plant, this behavior may be associated with crassulacean acid metabolism, in which the higher  $K^+$  levels provide stomatal opening (Debeaufort et al. 2000). Associated with this, the storage of organic acids in the vacuole at night is greater (Niechayev et al. 2019), being this the time that the harvest was carried out.

In view of the presented results, the choice of the harvest time will depend on the application and/or use of the mucilage. For example, in mucilage, the presence of electrolytes is important for the formulation of suspensions (Gebresamuel & Gebre-Mariam 2012), since the electrical conductivity directly influences viscosity, which influences the film production (i.e., biofilm). Thus, it is important to consider that changes in the harvest time will result in important electrolytic changes in the mucilage, which may interfere with its application.

There were significant differences in the pH of the mucilage with and without the addition of citric acid (Figure 2D). The PC1 accounted for 69.4 % of the data variance, with higher loads for the variables TA (0.96), SS (0.94) and EC (0.83), and also with a negative correlation between them and the pH (-0.95). The PC2, with 20.1 %, expressed the effect of the variable  $K^+$ , with a load of -0.98.

The addition of citric acid in the extraction reduced the pH to values close to 3 (data not shown). It is known that the ideal pH for film formulation is between 5.6 and 7, because, in this range, there is a spreading of the molecular configuration of the mucilage, due to the reduction of repulsion forces and a greater number of intermolecular hydrogen bonds, resulting in a more orderly three-dimensional network to produce compact and resistant films (Espino-Díaz et al. 2010). However, it is not yet possible to confirm the effect of organic acids on the mucilage composition, as it depends on its industrial application. However, a pH below 5, as observed in the present study, could hinder, for

example, its application as edible films. In addition, the neutral pH of the cactus favors the composition of pharmaceutical and food products (Kalegowda et al. 2017).

A well-balanced pH of the mucilage helps to lessen degradation in the conservation process and reactions with amino acids (Otálora et al. 2019). The results suggest that mucilage is a polyelectrolyte loaded with negative factor molecules. According to Trachtenberg & Mayer (1980), a negative charge produces strong intermolecular repulsion and, therefore, a more expanded molecule, which may result in high mucilage viscosity. On the other hand, the addition of positive ions reduces the repulsion and expansion of the molecules, producing a significant reduction in viscosity.

In the evaluation of the components for the cladode size groups, the dispersion of the scores was observed in Figure 2E. The PC1 represented 30.6 % of the data variance, characterized by a greater expression of the variables  $K^+$ , TA and SS, with loads of -0.71, -0.79 and -0.59, respectively. The PC2, with 25.8 %, is represented by the variation in pH (-0.85), and also SS (-0.65), a variable that presented contribution to both components. Together with the PC3 (data not shown), this added a total variance of 76.5 %, with eigenvalues  $> 1.0$ . The variable with the greatest contribution in the PC3 was the EC, with a load of 0.98.

Smaller cladodes are found, proportionally, in greater quantity in the plants. This allows a greater accessibility to the raw material for industrial use. The use of larger plants (231-300 mm; size L) exposes cladodes for longer periods to biotic and abiotic factors, which may compromise the quantity and quality of the raw material. The present study showed that the size L presented higher levels of SS, TA and  $K^+$ , which may, for example, make it difficult to form films. Thus, the size was also an important source of variation in the phytochemical composition of cladodes, and may also influence mucilage applications in the food industry.

Therefore, as observed in the four trials, the imposition of field management (i.e. cultivation conditions, harvest time and cladode size), as well as the addition of citric acid for mucilage extraction, resulted in significant changes in the studied physicochemical properties. This shows that these factors change the mucilage quality and that, depending on the application, may be favorable or

not for a certain purpose in the industry. Finally, the data reveal the importance of systematic handling and standardization of the raw material, especially with regard to environmental conditions and its processing for industrial purposes.

This study reports the first evidence of the importance of the environmental management of cacti, *Nopalea* genus, for the efficient use of its vegetative structures (cladodes), since management decisions alter the mucilage yield and chemical composition. Cacti are extremely adaptable to environmental conditions, resulting in significant physical-chemical changes which may enhance their application as food ingredients. In addition, the form of mucilage extraction in the present study was with water, a more economical and sustainable, environmentally friendly way, differently from other extractions in which organic solvents are used (Rodríguez-González et al. 2014, Sandoval et al. 2019). Thus, it is possible to replicate the extraction method for studies with other conditions affecting the plant, such as fertilization and intercropping.

Finally, other aspects must be taken into account when choosing the most relevant variable for obtaining mucilage. One is that, for semi-arid conditions, irrigated cultivation is expensive, and, in this region, water conditions are deficient, with low quality saline water. In addition, the night harvest time may be considered an inappropriate work shift. In addition, the larger cladodes may be difficult to handle. The time of the material in the field to reach the desired size also increases the chances of injury. In summary, these aspects should be considered, especially when it comes to the food industry, since these problems may lead to higher expenses and loss of time in the process of obtaining mucilage.

## CONCLUSIONS

1. The night harvest and rainfed cultivation increased some studied solutes, such as  $K^+$  and soluble solids content, as well as resulted in higher pH values. The rainfed plants also had mucilage with high electrical conductivity;
2. The addition of citric acid in the extraction of mucilage resulted in higher values of soluble solids (SS), titratable acidity (TA) and electrical conductivity, and a significant reduction in pH. The larger cladode size (231-300 mm) resulted in mucilage with higher levels of  $K^+$ , TA and SS.

*Nopalea* cladodes grown under irrigation showed a greater mucilage yield.

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