









Preparation and characterization of a biodegradable film from cactus *Nopalea* sp.

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Abstract. The increasing use of natural biodegradable polymers is a consequence of the concern of society with sustainability. Mucilage of forage cactus of the genus *Nopalea* is an attractive polymer matrix for the composition of edible films. Most studies on biodegradable films are carried out with the genus *Opuntia*. This makes *Nopalea* an important source of interest in the investigation of film formation. The objective was to study the formulation of films and evaluation of physical, optical, and mechanical characteristics of clones of *Nopalea* and *Opuntia*, through the addition of water and glycerol. The mucilage was extracted from cladodes of *Nopalea cochenillifera* Salm Dyck: clones IPA Sertânia (IPA) and Miúda (MIU); and, from *Opuntia stricta* [Haw.] Haw., clone Orelha de Elefante Mexicana (OEM). The powdered mucilage was hydrated and vacuum filtered, followed by the formulation of the films, without glycerol (just water) and with the addition of glycerol at 15%, 25%, and 14% for IPA, MIU, and OEM clones, respectively. The yield of powdered mucilage was higher for *Nopalea*. Optical tests with the films revealed that the addition of glycerol generated slightly yellow films (positive b^* values), with less transparency than the control films, regardless of the clone and higher L^* value in IPA clone films. Moisture content and thickness were increased when glycerol was added. All films, with and without glycerol, showed high water solubility. The microstructure revealed that the films with glycerol presented more compact, smooth, and linear surfaces, forming a homogeneous network. The FTIR spectra revealed that the glycerol films showed the same profile as the control samples, but with higher absorption intensities in some bands. The results found in the present work evidence the potential of the genus *Nopalea* to produce biodegradable films, as already widely known for the genus *Opuntia*.

Keywords: *Edible film; Fourier Transform Infrared Mucilage; Nopalea cochenillifera; Opuntia stricta; scanning electron microscopy.*

Introduction

Conventional plastics have extensive applications throughout the economy, such as in industry, commerce, and agriculture, with an essential role in food conservation through packaging (Díaz-Montes and Castro-Muñoz, 2021). On a global scale, the production of synthetic plastics has grown significantly over the last decade; with an estimated 370 million tons of plastics being produced in the year 2020 (Statista, 2022). The use of these petroleum-derived products has become indispensable in everyday life, as in addition to being practical, they are resistant, flexible, generally impermeable to vapors and water, and highly durable (Heidbreder *et al.*, 2019). On the other hand, they degrade slowly, contaminating

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water resources, soils, air, and foods, which can damage ecosystems and human Health (Paidari et al., 2021; Zhang et al., 2021). Thus, seeking to reduce the problems arising from the massive use of plastics, especially in food, the development of biomaterials has been encouraged as ecologically viable, profitable, and biodegradable alternatives from renewable sources (Otoni et al., 2017; Dubey and Dubey, 2020; Nanda et al., 2021). The use of biopolymers appears as an advantage as they can be used in several branches of industry, whether for pharmaceutical packaging, agricultural applications, and easily digestible coatings for food and without harm to health (Ali and Ahmed, 2018; Nanda et al., 2021).

The main sources of raw material for biopolymers are organic products of plants or animal origin (Otoni et al., 2017; Kumar et al., 2021). These must be able to provide a polymeric network with numerous intermolecular bonds to form a matrix, whether they consist of polysaccharides, proteins, fats, or even a combination thereof (Nandane and Jain, 2018; Ju et al., 2019; Lee et al., 2020; Espino-Manzano et al., 2020; Kumari et al., 2021; Chen et al., 2021). In fruit and vegetable conservation, the use of plant polysaccharides as edible coatings is widespread, especially in starch-based films due to their low acquisition cost, flexibility, and strength (Thakur et al., 2019; Tarique et al., 2021). Other plant by-products that present a diversified composition of biopolymers can also be considered an important means for the formation of edible films and coatings. Some studies have already demonstrated the potential of mucilage extracted from cacti, especially forage cactus, as having biopolymeric attractivity for the composition of edible films and coatings, as well as for pharmaceutical and cosmetics uses (Rodríguez-González et al., 2014; Gheribi et al., 2019; Morais et al., 2019; Otálora et al., 2021).

The cacti of the *Opuntia* and *Nopalea* genera, commonly known as forage cactus, are plants that present expressive morpho-anatomical adaptations, such as succulence, and due to Crassulacean Acid Metabolism (CAM), can grow and survive under severe drought conditions, characteristic of arid and semi-arid regions (Scalisi et al., 2016; Ventura-Aguilar et al., 2017). The parenchyma composition of the cladodes of *Opuntia ficus-indica* (L.) Mill. contains mucilage with several polysaccharides (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) and fibers, making it an important hydrocolloid with coating functionality for the industry (Sáenz et al., 2004; Ginestra et al., 2009). Due to its composition, forage cactus mucilage has appreciable nutritional and health attributes, in addition to having high thermal stability, as well as excellent thickening and gelling capacity (Otálora et al., 2021), making it a candidate for use in coatings (Morais et al., 2019) or edible films (Gheribi and Khwaldia, 2019a).

Most studies on film formulation are mainly focused on mucilage of the species *O. ficus-indica* (Espino-Díaz et al., 2010; Rodríguez-González et al., 2014; Gheribi et al., 2018; Du Toit et al., 2019; Gheribi and Khwaldia, 2019b;), with incipient data on cactus *Nopalea cochenillifera* Salm Dyck, a clone widely used in agriculture in the Brazilian semiarid region for animal feed purposes. In works with *Nopalea* mucilage, Morais et al. (2019) reported the use of *N. cochenillifera* mucilage biocoatings associated with the maintenance of sensory attributes and lower loss of fresh mass of minimally processed yam (*Dioscorea* spp.). Panta-Araújo et al. (2021) also found that the mucilage of the *N. cochenillifera* (IPA Sertânea and Miúda clones), showed similar physicochemical characteristics to the mucilage of *Opuntia stricta* [Haw.] Haw. (Orelha de Elefante Mexicana). In addition, the hydrated mucilage of *N. cochenillifera* (Miúda clone) preserved the attributes of proteins, total soluble solids, vitamin C, and electrical conductivity over a period of 12 days, in relation to the *O. stricta* (Panta-Araújo et al. 2021).

This suggests that mucilage extracted from the genus *Nopalea* is a likely candidate for the formulation of biodegradable films.

For the formation of films from forage cactus, the incorporation of plasticizers into the medium is required, since the mucilage itself becomes brittle and unstable as a film (De Farias *et al.*, 2021). It is necessary to incorporate plasticizing agents into the mix, in order to guarantee strength and malleability, thus intervening with the physicochemical, mechanical, thermal, and structural properties of edible films (Jouki *et al.*, 2013; Gheribi *et al.*, 2018). The main plasticizers for the formulation of cactus biopolymers are glycerol (Jouki *et al.*, 2013; Gheribi *et al.*, 2018), sorbitol (Gheribi *et al.*, 2018), combinations of glycerol and starch (De Farias *et al.*, 2021), chitosan and starch (Dominguez-Martinez *et al.*, 2017) and citrus pectin (Guadarrama-Lezama *et al.*, 2018; Sandoval *et al.*, 2019). In all these works, the results have demonstrated that the action of these plasticizers has a positive impact on the properties of thickness, transparency, tensile strength, water vapor permeability, stability, and solubility of the films. Glycerol is widely used in the formulation of edible films because it has a low molecular weight, a hydrophilic nature, capable of making chemical bonds with polysaccharides, thus reducing the forces of attraction between the polymers of the film matrix (Mali *et al.*, 2005; Antoniou *et al.*, 2014; Damas *et al.*, 2017).

The present study enriches the knowledge about the formulation of biodegradable films, having as the main raw material the mucilage of cacti of the *Nopalea* and *Opuntia* genera, elaborated with glycerol. Little has been reported in the literature on this using the *Nopalea* genus. An investigation of the physical-chemical, morphological and microstructural attributes of these cactus mucilage-based films were carried out.

Material and Methods

Plant material and climatic conditions

Cactus plants were cultivated in February 2016, in the experimental area of the Reference Center for Cactus Agrometeorology Studies and other Forage Plants at the Federal Rural University of Pernambuco (UFRPE/UAST), located in the municipality of Serra Talhada, Pernambuco, Brazil (7°59' S; 38°15' W and 431m), classified as a BShw climate region (Alvares *et al.*, 2013). The physicochemical characteristics of the cultivated soil have been described by Araújo *et al.* (2021). Three clones of forage cactus were cultivated: Orelha de Elefante Mexicana (OEM; *Opuntia stricta* [Haw.] Haw.), Miúda (MIU; *Nopalea cochenillifera* Salm Dick), and IPA Sertânia (IPA; *Nopalea cochenillifera* Salm Dick) spaced 1.0 x 0.2 m apart, with a total of 8 plants per useful plot for each clone. Cultural practices and fertilization management are described by Panta-Araújo *et al.* (2021).

Mucilage extraction

Second and/or third order cladodes of forage cactus were selected and harvested in November 2019, at 6 am and taken to the laboratory for weighing and mucilage extraction. An average of 6.5 ± 0.2 kg of cladodes was collected for each genotype. Mucilage was extracted according to Gheribi *et al.* (2018), with adaptations; this consisted of removing the peel (most external portion, epidermis, and chlorenchyma) of the cladodes to obtain the parenchyma (most internal part). The parenchyma was sliced into small cubic pieces and homogenized with the aid of a food processor (Philips alita, ri7775 Brazil) for 60 s, with 99.5% ethanol, at a ratio of 2:3 m/v (two parts of parenchyma to three parts of ethanol). The mucilage was obtained by filtering the alcohol present in the extract through

polypropylene fabric. The material obtained was dried in a forced air circulation oven at 55 °C for 24 hours. Then, the mucilage was weighed and stored for 12 days at 25 °C for film formulation.

Film formulation

The films were formulated according to Gheribi *et al.* (2018), with adaptations. Initially, 4 g of powdered mucilage were weighed and hydrated with 100 mL of distilled water. Then, the suspension was filtered using a vacuum pump with the aid of a paper filter. For the addition of the plasticizer to the filtered material, tests were carried out varying the concentrations of glycerol IMPEX® (% w/w), according to Gheribi *et al.* (2018), considering the formula:

$$GM(g) = \frac{[(GP) * (MM)]}{100} (1)$$

Where, GM = Glycerol mass (g); GP = glycerol percentage (%) and MM = mucilage mass. After the addition of glycerol to the filtered material, the solution was heated to 45 °C and stirred for 10 min. A 25 mL aliquot of each formulation was placed in Petri dishes and taken to a drying oven at 55 °C for 24 hours. Due to the intrinsic characteristics of the mucilage of each material, the ideal concentration of glycerol for film formation was variable for genotype. The criterion for choosing each concentration was the formation of a stable film that maintained its integrity after its removal from the petri dish and that did not present high humidity and stickiness that could harm the maintenance of the biopolymer. Therefore, the ideal concentrations of glycerol for the formation of the films of each clone were 15% for IPA clone, 25% for MIU, and 14% for OEM. For the controls, distilled water was added instead of glycerol to form the films and the material was dried at 55 °C for 24 hours.

Agro-industrial yield of powdered mucilage

The extracted dry mucilage was weighed to obtain yields in relation to the fresh weight of the whole cladode (MC) and of the parenchyma without the peel (epidermis + chlorenchyma) (MP). The income equation is given by:

$$MY (\%) = \frac{MPM}{MC} \times 100 (2)$$

$$MY (\%) = \frac{MPM}{MP} \times 100 (3)$$

Where MY is the mucilage yield (%); MPM = Powdered mucilage mass (g).

Color, transparency and visual assessment

The color was obtained through a colorimeter (RS-232 with serial output RGB-1002) with values obtained in the RGB system. The data obtained by the colorimeter were converted into the CIE L^* , a^* , b^* color scale (de Alvarenga Pinto Cotrim *et al.*, 2016). Where L^* corresponds to variations in the luminosity of the sample (0 to 100, darkest to lightest), a^* corresponds to variations from green (-a) to red (+a), and b^* is assigned to variations between blue (-b) to yellow (+b). The conversion of values was performed using online software available on a public website: <http://www.easyrgb.com/en/convert.php#Result>. Subsequently, the dataset of a^* and b^* was

converted and expressed in Chroma saturation values (C^*) according to the methodology of Espino-Días *et al.* (2010), where:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

Film transparency was determined by analyzing the absorbance of the films at 600 nm in a UV-VIS spectrophotometer (Biochrom, Libra S8, Cambridge, England) (Han and Floros, 1997). The films were cut and sized for entry into the glass cuvette. After reading, the absorbance values were converted into transmittance (% T) and applied to the equation:

$$T = \frac{\log T_{600}}{Th} \quad (5)$$

Where T is percent transmittance and Th is film thickness in mm. A qualitative analysis of the transparency of the films was also carried out through photographs with a digital camera (Nikon D3100). The films were placed on a paper surface that had a printed color image.

Thickness, moisture content and water solubility

The films obtained from each clone were submitted to the analysis of physical-chemical parameters. The thickness of the films was obtained according to Gheribi *et al.* (2018) using a micrometer accurate to 0.01 mm (Konica Minolta C224) and with 10 measurements at different positions along the film. The moisture content was determined according to Jouki *et al.* (2013): the films were cut into sizes of 2 x 2 cm, weighed, and left in an oven at a temperature of 55 °C until a constant weight was obtained; the moisture content was calculated according to the equation:

$$\text{Moisture content (\%)} = \frac{(M_i - M_f)}{M_i} \times 100 \quad (6)$$

Where M_i and M_f refer to the initial mass (g) before drying and final after drying, respectively. Water solubility was determined according to Jouki *et al.* (2013) where pieces of 2x2 cm were weighed, dried at 55 °C until reaching constant weight, and then immersed in 50 mL of distilled water at 25 °C for 30 min under stirring. The undissolved fragments were weighed and dried at 55°C until a constant mass was reached. Solubility was obtained through the following formula:

$$\text{Water solubility (\%)} = \frac{(M_i - M_f)}{M_i} \times 100 \quad (7)$$

Where M_i is the initial mass (g) of the film and M_f is the final mass (g) of the insolubilized film.

Scanning Electron Microscopy

The microstructural morphology of the surface of the films was evaluated by means of scanning electron microscopy (SEM), for which the samples were mounted in stubs and covered with gold using a metallizer brand DENTON VACUUM, model DESK V. Then, the samples were inserted into a TESCAN SEM, model VEGA3, with tungsten filament, where the images were obtained using an acceleration voltage of 20.0 kV.

Fourier Transform Medium Infrared Spectroscopy - FTIR

Film samples were subjected to spectral analysis in the mid-infrared region by Fourier transform (FTIR) (Frontier from Perkin Elmer®) with the aid of the universal accessory of attenuated total reflection (UATR). Absorbance was obtained in the spectra in the region of 4000-600 cm^{-1} , with a resolution of 8 cm^{-1} and 8 scans.

Experimental design and statistical analysis

The experimental design was completely randomized with four replications for each clone studied. Data were submitted to normality tests, analysis of variance (ANOVA), and Tukey test at 5% probability with the aid of the R x64 3.4.0 software. The graphs were developed using the Sigma Plot software, version 14.

Results and Discussion

Mucilage yield

The process of cutting the cladodes and separating the peel (epidermis and chlorenchyma) and parenchyma resulted in different mucilage yields (Table 1). Thus, in the evaluation of the yields of the entire cladodes and the parenchyma, only the clone MIU (*Nopalea cochenillifera* Salm Dick) obtained higher yields of fresh mass (Table 1). This highlights the potential of *Nopalea* clones for higher yields of mucilage extraction compared to *Opuntia* clones. In mucilage extractions derived from *Opuntia*, lower yields have been seen than those observed in the present study; e.g., 1.20% for *O. monacantha* (Dick *et al.*, 2019) and 0.68 % for *O. ficus-indica* (Espino-Díaz *et al.*, 2010). These variations in mucilage yield observed by these authors may be due to the extraction method used by them (aqueous extraction) when compared to the present work (alcoholic extraction). Factors such as the age and size of the cladodes, as well as the cultivation and climatic conditions, can alter the amount of mucilage extracted (Gheribi and Khwaldia, 2019a; du Toit *et al.*, 2020; Panta-Araújo *et al.*, 2021).

Table 1. Mucilage yield (%) of forage palm mucilage from IPA Sertânia and Miúda (MIU) (*Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (OEM) (*Opuntia stricta* [Haw.] Haw.) clones.

| Clones | Mucilage yield (%)* | |
|--------|---------------------|-------------|
| | Cladode | Parenchyma |
| IPA | 4.76 ± 0.79 | 6.39 ± 0.58 |
| MIU | 6.65 ± 1.39 | 8.98 ± 1.10 |
| OEM | 1.49 ± 0.13 | 3.11 ± 1.07 |

* Yield calculated based on total cladode fresh weight and relative to parenchyma. Values are mean ± standard deviation of the mean.

Optical properties of films

The addition of plasticizer to the mucilage of all the clones studied was necessary for the formation of more homogeneous films, as opposed to the control films (without plasticizer) (Fig. 1). These observations are relevant for the formation of a film with a hydrocolloid matrix, of which the use of plasticizer with hygroscopic properties increases the stability of the films. Espino-Díaz *et al.* (2010)

and Gheribi *et al.* (2018) report that glycerol has good characteristics as a plasticizer for cactus mucilage, as it provides high tensile strengths and low water vapor permeability. However, other organic plasticizers such as sorbitol and polyethylene glycol (PEG 400) have been characterized as cactus films due to higher tensile strengths and thermal stability than glycerol but being inflexible films in relation to glycerol. (Gheribi *et al.* 2018). Furthermore, considering that the mucilage polymer matrix of each genotype studied here has a distinct physicochemical nature, as reported by Panta-Araújo *et al.* (2021), the interactions between water molecules and polysaccharides are modified. Thus, the addition of glycerol as a plasticizer was modified in its concentration for each clone studied, since these physical-chemical attributes inherent to the mucilage of each clone were a network of differentiated interactions in the biopolymer solution. Regardless of which clone was used, the transparency values were higher for the control films (Fig. 1, A, D e G), as can be seen by a visual evaluation, the differences are smaller (Fig. 1 B, C, E, F, H e I). Lower transparency values for biodegradable films may be advantageous for food packaging since this film is able to reduce UV light incidence and prevent oxidative damage (Lee *et al.*, 2020). This is positive for commercial packaging where visibility of the food within is desired.

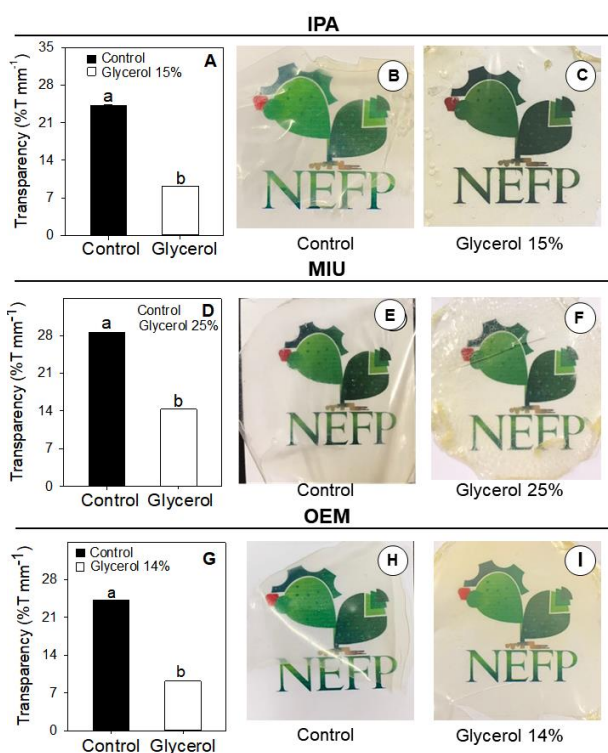


Figure 1. Transparency (%T mm⁻¹) and visual appearance of biodegradable films of forage palm, clones IPA Sertânia (A, B, C), Miúda (MIU) (D, E, F) and Mexican Orelha de Elefante (OEM) (G, H, I) without the addition of plasticizer (Control) and with the addition of glycerol plasticizer. The bars represent the standard deviation of the mean. Different letters correspond to statistical differences (Tukey test, $p < 0.05$).

Regarding luminosity (L^*), all films presented high values in general (Table 2). However, in the IPA clone films, the incorporation of glycerol increased the brightness compared to the control, while in the other clones, MIU and OEM, even with a slight glycerol increase, there were no significant statistical differences in relation to L^* (Table 2). Ahmadi *et al.* (2012) and Jouki *et al.* (2013a; 2013b) report that

increasing the concentration of plasticizers has an influence on the L^* values of films, making them brighter. This fact is also corroborated by Makhloufi *et al.* (2021), who registered a direct effect of glycerol concentration on L^* values. On the other hand, the addition of glycerol resulted in films with higher C^* values (Table 2).

Table 2. Color in standard Luminosity (L^*), a^* , b^* and Chroma (C^*) of biodegradable films based on forage palm mucilage from clones IPA Sertânia and Miúda (MIU) (*Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (OEM) (*Opuntia stricta* [Haw.] Haw.), without the addition of plasticizer (Control) and with the addition of glycerol plasticizer.

| Clones | Luminosity (L^*), a^* , b^* and Chroma (C^*) | | |
|--------|--|------------------------------|------------------|
| | | Control | Glycerol |
| IPA | L^* | 85.726 ± 1.04 b [‡] | 94.695 ± 0.91 a |
| | a^* | -0.8010 ± 0.44 a | -3.311 ± 0.91 a |
| | b^* | 0.9175 ± 0.06 b | 10.217 ± 2.38 a |
| | C^* | 1.6350 ± 0.51 b | 10.746 ± 2.54 a |
| MIU | L^* | 85.169 ± 1.44 a | 90.398 ± 1.63 a |
| | a^* | 1.3760 ± 0.05a | -1.5313 ± 0.59 b |
| | b^* | -2.154 ± 0.66 b | 5.5193 ± 0.22 a |
| | C^* | 2.5987 ± 0.33 b | 5.7941 ± 0.22 a |
| OEM | L^* | 82.708 ± 1.70 a | 84.279 ± 0.21 a |
| | a^* | 0.777 ± 0.46 a | -2.017 ± 0.12 b |
| | b^* | 4.321 ± 0.89 a | 7.696 ± 0.81 a |
| | C^* | 3.8029 ± 0.79 b | 7.9573 ± 0.81 a |

[‡] Values are mean ± standard deviation of the mean. Different letters correspond to statistical differences (Tukey test, $p < 0.05$).

In relation to the a^* values, a tendency towards more negative values was noted with the addition of glycerol for all clones, even though no significant differences were observed for the films of the IPA clone (Table 2). On the other hand, the b^* values were more positive when glycerol was added in relation to the control, even though no significant differences were observed at the OEM, indicating that the films presented a more yellowish coloration. In general, the increases in C^* values correspond to the increase in the color saturation of the films based on the *O. ficus-indica* mucilage, associated with a more compact and smoother microstructure of the films with glycerol (Espino-Díaz *et al.*, 2010). In the present work, the presence of the plasticizer glycerol produced films with an opaquer appearance and a more yellow-greenish tint was seen to be associated with higher negative a^* and more positive b^* values. This fact was reported by Damas *et al.* (2017), where they verified a yellow tendency in *C. hildmannianus* films with high glycerol concentration. Similarly, in films made with chia seed mucilage, the addition of increasing concentrations of glycerol increases the yellowish coloration of the films (Dick *et al.*, 2015). The qualitative transparency of the films in Fig. 1 B, C, E, F, H, and I, reveal subtle differences, supporting the idea that the biodegradable films produced in this work are attractive candidates for edible coatings.

Physical-chemical properties

The addition of glycerol significantly increased the moisture content independent of the clone studied (Table 3).

Table 3. Moisture (%) and water solubility (%) of edible films based on forage palm mucilage from clones IPA Sertânia and Miúda (MIU) (*Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (OEM) (*Opuntia stricta* [Haw.] Haw.), without the addition of plasticizer (Control) and with the addition of glycerol plasticizer.

| Clones | Film moisture (%) | | Solubility in water (%) | |
|--------|-------------------|----------------|-------------------------|----------------|
| | Control | Glycerol | Control | Glycerol |
| IPA | 2,18 ± 0,83 b* | 9,51 ± 1,19 a | 96,05 ± 3,19 a | 98,17 ± 1,80 a |
| MIU | 0,74 ± 0,07 b | 14,94 ± 2,22 a | 92,78 ± 5,78 a | 89,59 ± 5,24 a |
| OEM | 1,88 ± 0,18 b | 9,53 ± 2,75 a | 94,96 ± 5,61 a | 96,04 ± 2,46 a |

* Values are mean ± standard deviation of the mean. Different letters correspond to statistical differences (Tukey test, $p < 0.05$).

On the other hand, increases in solubility were not significant (Table 3). Due to the hydrophilic nature of glycerol, there is greater retention of water in the films, as observed by Zhang *et al.*, (2016), Gheribi *et al.* (2018), and Tarique *et al.* (2021), evidenced also in the present work. Glycerol has hygroscopic characteristics, due to the presence of free hydroxyl groups and the formation of hydrogen bridges with the hydrophilic structure of the mucilage, consequently, it is able to retain water easily (Cerqueira *et al.*, 2012; Zhang *et al.*, 2016; Syafiq *et al.*, 2022). The water solubility parameter of films is a critical point for food applications, given that high solubility values are highly effective for use in fresh and processed foods (Tarique *et al.*, 2021). Guadarrana-Lezama *et al.* (2018) found that low concentrations of mucilage (5%) from the *O. ficus-indica* palm provided more soluble films compared to higher concentrations (up to 20%); this is because the saccharides have more hydrogen bonds with the water molecules in the surrounding medium. Gheribi *et al.* (2018) observed that films based on *O. ficus-indica* mucilage (4%) and glycerol (40%) showed 58% water solubility. Due to its hydrophilic properties, an increased concentration of the plasticizer glycerol can gradually increase the water solubility of the films (Razavi *et al.*, 2015; Zhang *et al.*, 2016; Makhouloufi *et al.*, 2021; Tarique *et al.*, 2021). The addition of glycerol to the mucilage resulted in thicker films, regardless of the clone studied (Table 4).

Table 4. Thickness (mm) of edible films based on forage palm mucilage from clones IPA Sertânia and Miúda (MIU) (*Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (OEM) (*Opuntia stricta* [Haw.] Haw.), without the addition of plasticizer (Control) and with the addition of glycerol plasticizer. Values are mean \pm standard deviation of the mean.

| Clones | Thickness (mm) | |
|--------|----------------------|---------------------|
| | Control | Glycerol |
| IPA | 0,075 \pm 0,019 b* | 0,205 \pm 0,037 a |
| MIU | 0,071 \pm 0,02 b | 0,215 \pm 0,030 a |
| OEM | 0,065 \pm 0,01 b | 0,118 \pm 0,035 a |

* Different letters correspond to statistical differences (Tukey test, $p < 0.05$).

This resulted in less brittle and more visually intact films (appearance evaluation). Due to the capacity of glycerol to occupy more empty spaces and allow more bonds with the polymeric matrix of the mucilage, generally, its addition gives the films a greater thickness (Jouki *et al.*, 2013; Tarique *et al.*, 2021), which changes the film's mechanical properties and serves to impede the passage of gases and water vapor. Espino-Díaz *et al.* (2010), Damas *et al.* (2017) and Gheribi *et al.* (2018) observed that adding a plasticizer to the films with glycerol also increases the thickness by about 0.10 to 0.18 mm in all cases, similar to findings in the present work. Clones of the genus *Nopalea* enabled thicker plasticized biodegradable films, compared to the genus *Opuntia* (Table 4). This characteristic suggests satisfactory durability of the polymer and its applicability as an edible coating since this biopolymer would possibly last longer on the surface of fruits or vegetables for commercialization. However, the purpose of the films depends on their general characteristics, and the moisture content and solubility, on the whole, should be evaluated.

Microstructural characteristics of the films by SEM

From the surface microstructure of the films obtained by SEM, it was qualitatively verified that the films of all palm clones formulated without the addition of plasticizer showed inhomogeneous particles and circular structures, such as agglomerates of particles with some joined on their surface (Fig. 2 A-C).

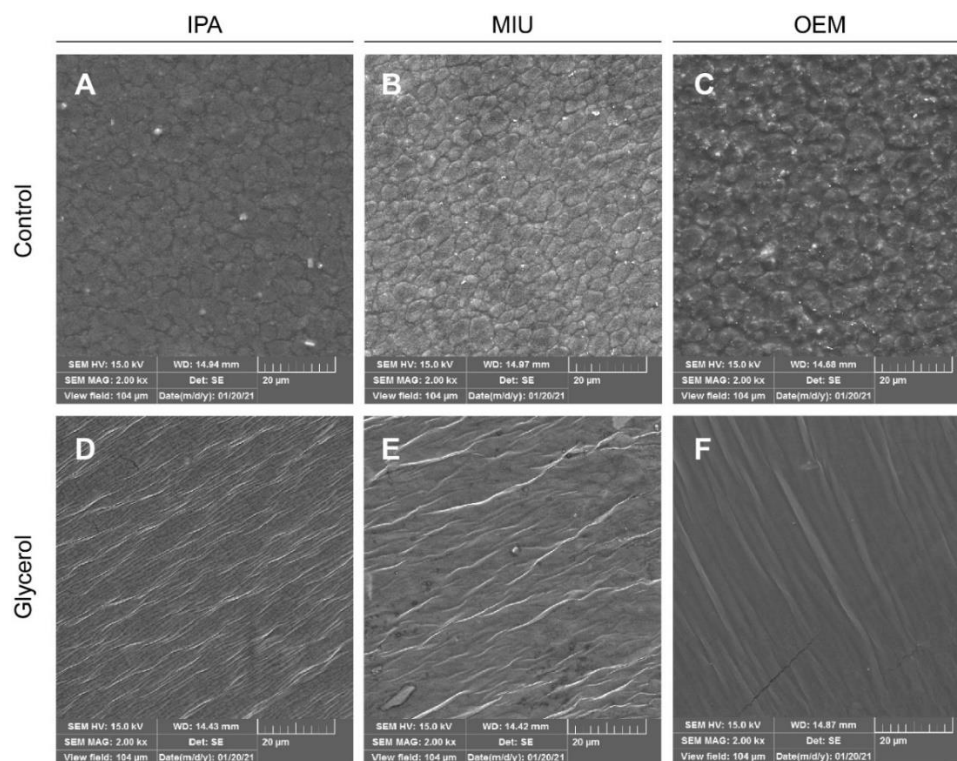


Figure 2. Scanning electron microscopy of the surface of edible films of forage palm clones IPA Sertânia (A, B), Miúda (MIU) (C, D) and Orelha de Elefante Mexicana (OEM) (E, F) without the addition of plasticizer (Control) and with the addition of glycerol plasticizer. (Magnitude 86x).

This is possibly related to those particles which are highly cohesive, such as potassium and calcium salts, as well as other components of the polymeric matrix. In contrast, the films made with glycerol were more compact, smooth, and had linear surfaces compared to the controls, forming a homogeneous network (Fig. 2 D-F). These results were similar to the films of *Cereus hildmannianus* K. Shum cactus, obtained by Damas *et al.* (2017), in which there were no mucilage residues in the plasticizer treatments, but the controls (without plasticizer) showed non-solubilized mucilage particles on their surfaces. Glycerol as plasticizer and water together reduce the intermolecular bonding forces between the mucilage constituents, granting the film less dense and softer characteristics (Guadarrama-Lezama *et al.*, 2018). On the other hand, the method of extraction and the use of mucilage can interfere with the structural quality of the films. De Farias *et al.* (2021) when looking at SEM results, found that the addition of "cactus flour", *O. ficus-indica*, (another method of obtaining mucilage) was not efficient in forming a film with starch and glycerol.

Fourier Transform Infrared Spectroscopy (FTIR)

The spectroscopic data in the mid-infrared region of all samples showed the same spectral profile as reported by Damas *et al.* (2017), Gheribi *et al.* (2018), and Otálora *et al.* (2021) (Fig. 3). However, differences in the intensities of some bands were observed between the control films and those with glycerol, independent of the clone evaluated (Fig. 3). The bands between 3400 and 3200 cm^{-1} correspond to O-H vibrations of alcohol and carboxylic acid groups (-C(O)-OH) responsible for hydrogen bridges and intramolecular OH bonds, common features of bonds with water molecules (Pavia *et al.*, 2008). Another highlight for differences between control and added glycerol treatments

was the higher absorption between 2932 cm^{-1} and 2888 cm^{-1} , designated as C-H stretching and portions of carboxylic acids and aldehydes (Pavia *et al.*, 2008; Silverstein *et al.*, 2014). A peak at 1722 cm^{-1} appears, assigned to carbonyl stretching ($\text{C}=\text{O}$), characteristic of films with the addition of plasticizers (Gheribi *et al.*, 2018) and mucilage powder of the species studied in this work (Araújo *et al.*, 2021). In addition, the band 1610 cm^{-1} is assigned to COO^- (carboxylate ion) (Rodríguez-González *et al.*, 2014). A set of bands with absorbances between 1408 and 1247 cm^{-1} can be assigned to the C-H or -OH bonds of alcohol groups (Choque-Quispe *et al.*, 2021). Furthermore, a higher intensity was observed in the band around 1047 cm^{-1} , observed as C-O stretching corresponding to alcohols, carboxylic acids, esters, and ethers (Pavia *et al.*, 2008; Silverstein *et al.*, 2014). This type of vibration in the films indicates bonds between carbohydrate molecules present in the mucilage (Ayquipa-Cuellar *et al.*, 2021). According to Guadarrama-Lezama *et al.* (2018), the bands with lower frequencies up to 1200 cm^{-1} are characteristic regions of the presence of mucilage polysaccharides, but with intense complexity of interactions, which makes the specific identification of these sugars difficult.

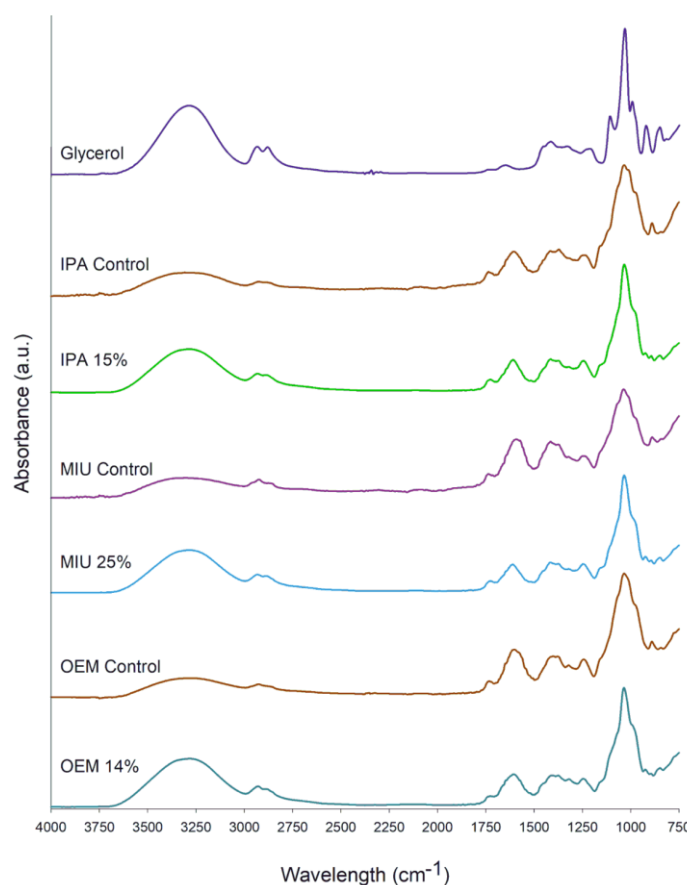


Figure 3. FTIR spectra of glycerol and forage palm films of clones IPA Sertânia, Miúda (MIU) and Orelha de Elefante Mexicana (OEM) without the addition of plasticizer (Control) and with the addition of glycerol plasticizer.

Conclusions

The yield of powdered mucilage was higher for *Nopalea*, compared to *Opuntia*. Optical tests with the films revealed that the addition of glycerol generated slightly yellow films (positive b^* values), with less

transparency than the control films, regardless of the clone and higher L^* value in IPA clone films. Moisture content and thickness were increased when glycerol was added. All films, with and without glycerol, showed high water solubility. The microstructure revealed that the films with glycerol presented more compact, smooth, and linear surfaces, forming a homogeneous network. The FTIR spectra revealed that the glycerol films showed the same profile as the control samples, but with higher absorption intensities in some bands. Therefore, films from the *Nopalea*, with glycerol, presented physical attributes such as transparency, color, microstructure, and visual appearance compatible with *Opuntia* clone films. The results found in the present work evidence the potential of the genus *Nopalea* to produce biodegradable films, as already widely known for the genus *Opuntia*. This fact makes the cultivation of *Nopalea* attractive due to its social and economic importance to the countries that cultivate it.

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Statement of ethics

Not applicable

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Consent to publication

Not applicable

Data availability

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

Conflict of interests

The authors declare that they have no conflict of interest.

Author contributions

FALB: Conceptualization, methodology, software, formal analysis, investigation, data Curation, writing - original draft. KSF: conceptualization, methodology, formal analysis, writing - review and editing, supervision, project administration. AMSSB: conceptualization, methodology, formal analysis, writing - review and editing, supervision, coordination of infrared analysis. YPA: Methodology and investigation. ASANM and NLF: Methodology and investigation. TGFS: conceptualization, methodology, formal analysis, writing - review and editing, supervision, coordination of cactus planting. ANS: conceptualization, methodology, writing - review and editing, visualization, supervision, project administration, funding acquisition.

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