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Research Article

Extraction and characterization of mucilage from *Opuntia ficus-indica* cultivated on hydroponic system

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Abstract

An interesting component of *Opuntia ficus-indica* is the mucilage for its properties and industrial uses. However, the great variability of its quantity and quality caused by different growing conditions, the hydroponic system is an alternative. The objective of the present study was cultivating 4 species of Mexican Nopal in a hydroponic system, extract and characterize the mucilage. The characterization consists of pH, °Brix, colour, proximal analysis, phenols, antioxidant activity, crystallinity, and chemical bonding constituents. 'Copena F1' is the best alternative for production of biomass and mucilage. 'Villanueva' had high levels of phenols (1,311.83 mg GAE g^{-1}), antioxidant capacity $ABTS^{\bullet+}$ (6,301.12 mg TE g^{-1}) and FRAP (536.26 mg GAE g^{-1}). A large amount of lipids (1.39%), and nitrogen-free extract (49.27%). The functional groups of the mucilage were identified (-OH, -CH, $-CH_2$, $-CH_3$, C=C, HCH, -CHO) and gypsum, cellulose, SiO_2 $CaSO_4$, $C_2H_2K_2O_5$, $CaCO_3$ and CaH_2 by X-ray diffraction. The hydroponic system is a viable alternative for production of nopal and mucilage of high-quality mucilage that can be used in several sectors of the industry.

Keywords: antioxidants; mucilage; biomass; hydroponic

Introduction

In recent years, the interest of the homogeneity of crops has taken a big importance to increase the quantity and quality of food for providing clean and fresh foods for the next generations. One of the main alternatives is to establish crops under systems that allow controlling mainly the quantity of nutrients, increasing populations densities and without the need of large extensions of land. All this, cultivation, propagation, and conservation of several crops like Nopal relies on traditional agro practices techniques. However, these conventional practices did not fulfil the desired needs; as large-scale production (high value,

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low cost) of important crops and their intrinsically compounds (Thakur *et al.*, 2019). In the future, the additional pressure will be working on how we can more efficiently utilize the natural resources to produce foods. The natural resources include soil, water, air and how to use them in sustainability. The reasons behind of the unproductive, unfertile and unsuitable agriculture activities are from inadequate soil, management, urbanization, continuous cropping, frequent drought, less water management and the decrease in groundwater (Popp *et al.*, 2014; Lakhiar *et al.*, 2018).

Under such circumstances, the search of new farming technologies like a hydroponic system is a viable alternative. The hydroponic system is a technique to grow plants in nutrient solution (Lakkireddy *et al.*, 2012) without the soils (only an artificial support) in that the plant roots survive in a wholly immersed nutrient rich water (Lakhiar *et al.*, 2018). Some of the most important benefits included mitigation of environmental changes, increasing the global food production, efficient utilization of natural resources, is a soil-less technique, provide continuous enough, fresh clean and hygienic product supply throughout the year (Son *et al.*, 2016; Ndulini *et al.*, 2018). By this way, the Nopal is a cactus that is growing and harvested in arid and semi-arid regions worldwide with diverse industrial applications, is widely cultivated, consumed and Mexico is one of the first producers around the world for human consumption with 829,000 metric tons in 2017 (FAO, 2018; Statista, 2017). Nopal has been traditionally used for preparing diverse local foodstuffs, powder, nopal in pickled or brine, juice and syrup (Guadarrama-Lezama *et al.*, 2018).

Recently, several authors have evaluated the yield of Nopal from hydroponic system at different population densities, substrates, experimental designs, management of nutritive solutions, conditions and zones as Sonora, Morelos, Mexico City, Baja California, Jalisco, Oaxaca, and Nuevo León from 60 to 80 t ha-1 (SAGARPA-SIAP, 2007; Vázquez-Alvarado *et al.*, 2009; Rodríguez-Fuentes, 2009; SAGARPA, 2018). But the nopal it is not common to be produced in a hydroponic system since they are CAM plants, that is, they are those plants that have an acidic metabolism of the Crassulaceae that minimize photorespiration to the minimum and save water by separating these steps in time, between day and night. They are also characterized by having the stomata open during the night to produce malic acid (power of acidity), and they are better adapted to very hot and dry climates. Variables such as climate, species, age of the cladode (maturity), climatic season and the topography of the place of planting (type of soil, rain, temperature, etc.) interfere in the biomass of the cactus and therefore of the mucilage. The hydroponic nopal contain a thick white cuticle on the inside with cavities of scattered cellulose layers that make up the collenchyma and chlorenchyma, which make up the parenchyma, producing mucilage (Sandoval *et al.*, 2019). Among these tissues are cells with oxalate crystals (drusen) and soluble and insoluble fibers in an aqueous medium and when the greater the area and surface thickness of each cladode, the mucilage content will increase (Luna-Sosa *et al.*, 2019, 2020).

However, in the Nopal processing mucilage is separated and removed from products because the sap may confer undesirable textural and sensorial properties to the final product. The mucilage from the genus *Opuntia ficus-indica* is composed of heteropolysaccharide hydrocolloids (L-arabinose, 47%; D-xylose, 23%; D-galactose, 18%; L-rhamnose, 7% and D-galacturonic acid, 5%) with minerals such as calcium (4.15%), potassium (3.76%), sodium (0.36%), magnesium (0.81%), phosphorus (0.11%), zinc (3.64%), iron (21.07%), copper (1.56%) (Flores-Mendiola, 2012; Sangeethapriya and Siddhuraju, 2014) with a wide range of physicochemical properties. The color is yellow-green hue, pH slightly acidic, non-Newtonian shear-thinning behaviour (Contreras-Padilla *et al.*, 2016; Luna-Sosa *et al.*, 2020).

The cultivars studied, 'Copena F1' (CF1) produces long, thin, and green pencas, was developed in the College of Postgraduates-National School of Agriculture, Chapingo, Mexico (Mondragón-Jacobo and Pérez-González, 2003). *O. undulata* (Oa) is a morphospecies producer of tuna created in the School of Agronomy, UANL, 'Monterrey', is distinguished by its wavy margin, small and smooth spines and their surface extremely waxy (Gallegos-Vázquez *et al.*, 2006; Flores-Hernandez *et al.*, 2016). 'CF1' and 'Oa' are considered for a vegetable purpose. 'Jalpa' (Ja), produces semi-long and narrow buds while 'Villanueva' (Va) has a longitudinal semicircular shape. Both cultivars are from Zacatecas. 'Ja' and 'Va' are multipurpose cultivars (Luna-Sosa *et al.*, 2019). By this reason, the aim of this study was to evaluate the influence of a hydroponic system in the quality and quantity of produced mucilage by four cultivars. Likewise, to characterize the mucilage produced due to that no of a complete characterization of this material (mucilage) produced under hydroponic conditions.

Materials and Methods

Biological material

Four cultivars ('Villanueva', 'Jalpa,' '*O. undulata'* and 'Copena F1' - abbrev. 'Va', 'Ja', 'Ou' and 'CF1') were provided by the School of Agronomy, Campus Marín, UANL. The material was selected with the similar characteristics (height, thickness, and width) without visual damage.

Establishment of the crop

The hydroponic system (HS) was installed in the same School of Agronomy, Campus Marín, Nuevo León, Mexico (25° 23'N, 100°2'O, 393 masl). The bench had 1.1 m of wide and 14 m long. The floor and walls are of concrete. Tezontle was used as support (4 months of age). The nutrient solution (NS) composed by macro and microelements (200 N, 60 P, 250 K, 200 Ca, 50 Mg, 100 S, 0.50 Fe, 0.25 Mn,0.25 B, 0.02 Cu, 0.25 Zn and 0.01mg L^{-1} of Mo) with pre adjusted pH to 5.5 was used according to the proposed by Rodríguez-Fuentes *et al.* (2011). Four cultivars were placed in the HS with a completely randomized design with a population density of 30 plants m^{-2}. The irrigation of the crop was performed every 15 days with the NS.

Biomass of Opuntia ficus-indica

To know the accumulation of mucilage inside of cladodes in the period March-April 2018, the sampling of cladodes was taken at 45 days. For each treatment, the number and total fresh weight of the cut cladodes were determined. The cladodes were washed with distilled water and dried in a drying oven (Thermo Fisher Scientific, USA) at 75 ± 2 °C. The moisture content was calculated according to the reported by Almaguer-Sierra *et al.* (2014).

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Mucilage extraction

The extraction was carried out based on previous reports (Espino-Díaz *et al.*, 2010; Rodríguez-González *et al.*, 2014; Sandoval *et al.*, 2019) with some modifications. The cladodes were cut into small pieces, placed in distilled water (1:1 w/v) and mixed in a magnetic stirring plate (Thermo Fisher Scientific SP13163033Q, USA) for 10 min until obtain a homogeneous suspension. After that, was heated (110 °C/60 min) with constant agitation and centrifuged (Ultracentrifuge 5810/5810 R, Eppendorf, U.S.A.) at 252 x g/20 min. To precipice the mucilage, ethanol (98%) was added to the supernatant in a ratio 2:1 v/v and stored at 4°C/60 h. Ethanol was evaporated in a convention oven (Model ED 115, Thermo Fisher Scientific, USA) at 70 °C/2 days. The mucilage precipitated was pulverized and sieved (sieve No. 4, particle size of Ø 1.75 mm, MEQUIM, U.S.A.) to obtain a homogeneous particle size. The samples were stored in an airtight container until use. The yield was calculated according to the equation 1.

$$\%\,Yield = \frac{Dry\ weight\ obtained\ of\ mucilage\ (g)}{Fresh\ weight\ of\ extract\ (g)} \times 100 \qquad Eq.(1)$$

Mucilage's characterization

Physicochemical analysis

One g of mucilage powder was resuspended in 10 mL of water-methanol (1:1.75 v/v). The pH was measured with a calibrated pH meter (Hanna HI 2211, Germany). Total soluble solids (TSS) were determined with a 0-30% refractometer (Optika, DC-HR 130, China). The color was measured with a colorimeter (Minolta, CR-400, USA) and the results expressed based on the CIELAB profile, using the color coordinates L* (luminosity), a* (red/green) and b* (yellow/blue). The determinations were made in triplicate.

Proximal analysis

Proximal evaluations were made according to the recommendations of Official Analysis Method (AOAC, 1984). Moisture was carried out with the 934.06 test. Crude protein was analyzed with the Kjeldhal method (Model GL-44, USA) 960.52. Total fat according with the Soxhlet method (Model EV6 All, Gerhardt, Germany) 960.39. Ash content was calculated based on the incineration in a muffle (Model ECO-2L, USA) at 550 °C following the test 923.03. Crude fiber was evaluated with the enzymatic-gravimetric method (method 985.29). Nitrogen-free extract was analyzed qualitatively with the difference of 100% of the samples. The values obtained in percentages were converted with the angular transformation of Bliss through the results of arcsine in triplicate to be analyzed statistically.

Determination of total polyphenols content (TPC)

The total polyphenols content (TP) was determined by Folin-Ciocalteu method according to Georgé *et al.* (2005) and Hernández *et al.* (2018). Methanolic suspension of mucilage was prepared (1:20 w/v). Subsequently, 25 μL of mucilage suspension was mixed with 25 μL of Folin-Ciocalteu reagent, and after 1 min, 25 μL of sodium carbonate (75 g L^{-1}) was added. The solution was mixed thoroughly and incubated a 40 °C/30 min in a water bath. After that, the mixture was adjusted with 200 μL of distilled water and the absorbance was recorded at 750 nm with a microplate reader (Synergy HT Multi-Detection Microplate Reader, BioTek Instruments, USA). The absorbance of the sample was compared with the gallic acid standard curve to estimate the concentration of TPC in the mucilage suspension. The TPC were calculated as mean ± SD and expressed in mg of gallic acid per g of raw material (mg GAE g^{-1}).

Antioxidant activities

DPPH• radical scavenging activity

The antioxidant activity in the mucilage was evaluated as the DPPH• free radical-scavenging activity. This activity was estimated using the method proposed by Brand-Williams *et al.* (1995), Castro-López *et al.* (2017) and Rojas *et al.* (2018). The hydrogen atom or electron donation abilities of the sample was measured from light-purple colored DPPH methanol solution (60 mM). Subsequently, 5 μL of mucilage solution was added to a 295 μL DPPH• radical solution. After a period of incubation in the dark for 30 min, the absorbance was recorded at 517 nm with the same microplate reader. The ability to inhibit was calculated by the eq. (2), and expressed as percent inhibition of DPPH• radical:

$$\text{Inhibition (\%)} = \left[\left(A_{\text{Control}} - A_{\text{Sample}}\right)/A_{\text{Control}}\right] \times 100 \qquad \text{Eq. (2)}$$

Where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{Sample} is the absorbance with the mucilage solution. Gallic acid was used as reference. The DPPH• inhibition was expressed as the average of three replications in gallic acid equivalents in mg per gram (mg GAE g^{-1}) of raw material.

<u>$ABTS^{•+}$ radical scavenging activity</u>

The inhibition of the $ABTS^{•+}$ radical was made according to the methodology proposed by Hernández *et al.* (2018) and van den Berg *et al.*,1(999). The $ABTS^{•+}$ radical cation was generated by mixing an aqueous solution of potassium persulfate (2.45 mM) and $ABTS^{•+}$ (7mM). These reagents react stoichiometrically in a ratio of 1:2 respectively and must be kept in the dark at room temperature for 12 h before use. Diluted solutions of $ABTS^{•+}$ were prepared in ethanol until value of 0.700 ± 0.002 nm absorbance was obtained. Then, 95 μL of the dilute solution of $ABTS^{•+}$ was mixed with 5 μL of mucilage solution and the absorbance was measured at 734 nm with the same microplate reader. The capacity to inhibit the radical was calculated according to the eq. (3) and the results were expressed as percent inhibition of the $ABTS^{•+}$ radical:

$$\text{Inhibition (\%)} = \left[\left(A_{\text{Control}} - A_{\text{Sample}}\right)/A_{\text{Control}}\right] \times 100 \qquad \text{Eq. (3)}$$

Where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{Sample} is the absorbance with the mucilage solution. The $ABTS^{•+}$ scavenging ability of mucilage solution was calculated according to the standard curve plotted with Trolox and expressed as Trolox equivalent in mg per gram (mg TE g^{-1}) of raw material.

<u>Ferric reducing power assay (FRAP)</u>

The ferric ion (Fe^{3+}) reducing power was determined as described by Benzie & Strain, 1996 with a slight modification by Castro-López *et al.* (2017) and Bautista-Hernández *et al.* (2021). To 5 μL of a mucilage solution was added 12 μL of phosphate buffer pH 7. After that, it was added to the mixture 22 μL of potassium ferrocyanide 1%, homogenized and incubated in a boiling water bath at 50 °C/20 min. After cooling, 12 μL of trichloroacetic acid 10% was added. Also, 45 μL of distilled water and 10 μL of ferric chloride 0.1% were added and shaken thoroughly. The absorbance was recorded at 700 nm with the same microplate reader. Finally, the results were reported as gallic acid equivalents in mg per gram (mg GAE g^{-1}) of raw material.

Crystallinity and chemical bonding constituents

Crystallinity phases of the mucilage were evaluated by X-ray diffraction (XRD) according to the method reported by Ballesteros *et al.* (2018) using a D8 Discover diffractometer (Brunker, corporation) with the Cu tube (λ= 1.5406 Â). The radiation was generated at 25 mA and 30 kV. The scattering angle of 2θ from 10° to 60° at the step size of 0.04 and 1 s exposure at each step. Chemical groups and bonding arrangements of constituents present in the mucilage were determined by Fourier transform infrared spectroscopy (FTIR) using a Bunker Model FT-IR Vertex 80/80 v spectrometer (Boston, USA) in attenuated total reflectance mode (ATR) with a platinum crystal accessory. The measurements were recorded with a wavenumber range from 4000 to 400 cm-1 and 3 scans per sample.

Statistical analysis

All experiments were analyzed in triplicate (n = 3) and results expressed as mean ± standard deviations. Analysis of variance (ANOVA) was calculated with Tukey's test through the Statistica 21.0 software (IBM Corp., New York, NY, USA) at a 95% confidence level.

Results

Crop and yield

The yield and biomass production for the four cultivars examined varied significantly (Table 1). The cultivar with the highest biomass production was 'CF1' (317.58 ± 3.17 g m^2 DB), followed by 'Va', 'Ja' and 'Ou'

(77.94 ± 2.49, 75.37 ± 3.54 and 61.82 ± 2.35 g m^{-2} DB, respectively). Also, the yield of mucilage ranged from 8.74 to 16.47 % with the highest and lowest values being observed for 'Ja' and 'CF1' cultivars, respectively (Figure 1).

Table 1. Biomass production of different cultivars of hydroponic Nopal

Cultivar	Total fresh biomass (g m^{-2})	Total dry biomass (g m^{-2})
Copena F1	3,028.23 ± 29.96 [a]	317.58 ± 3.17 [a]
Villanueva	897.33 ± 35.15 [b]	77.94 ± 2.49 [b]
Jalpa	719.33 ± 25.89 [b]	75.37 ± 3.54 [b]
O. undulata	637.33 ± 23.00 [b]	61.82 ± 2.35 [b]

Different letters indicate significant difference (p≤0.05) between the cultivars (n = 3).

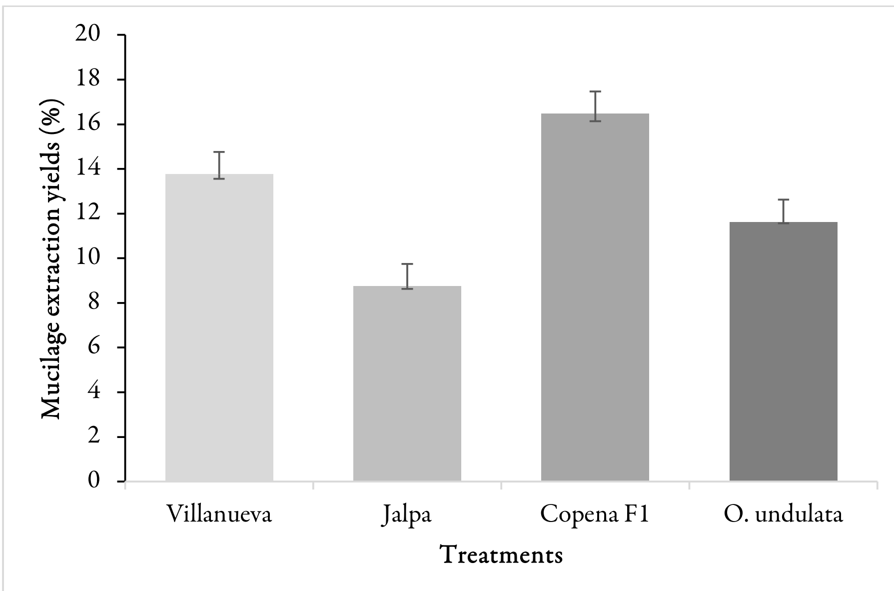

Figure 1. Yields of mucilage's extracted from different cultivars (45 days of maturity) grown in a hydroponic system

Different letters indicate significant difference (p≤0.05), n = 3

Physicochemical and proximal analysis

We examined the physicochemical and proximal composition of the four cultivars, including, pH, color and TSS (Tables 2, 3). pH values varied from 5.19 ± 0.00 to 5.58 ± 0.03. Among the four varieties the low value was found in 'CF1'. Significant differences (p ≤0.05 in TSS were found. 'Ja' presents the highest value (17.65 °Brix), followed by 'VA', 'Ou' and 'CF1' with 16.88, 16.05 and 15.31 °Brix, respectively. Significant differences were found (p ≤0.05) in the values of L*, a* and b* of the different mucilage's (Table 2). The highest brightness (L*) was presented by 'CF1' (81.54 ± 0.46), followed by 'Va' (72.85 ±0.05), 'Ja' (67.46 ± 0.03) and 'Ou' (65.20 ± 1.93). For a* value was -4-54 ± 0.05 (CF1), 0.54 ± 0.67 ('Va'), -2.29 ± 0.35 ('Ja') and 3.09 ± 0.19 ('Ou'). For b* value was 16.76 ± 1.00 ('CF1'), 23.31 ± 0.53 ('Va'), 20.87 ± 0.53 ('Ja') and 20.53 ± 3.20 ('Ou'). Table 3 shows the results obtained in proximal composition. The moisture content was 31.26 ± 0.25% ('Va'), 10.52 ± 0.09% ('CF1'), 6.05 ± 0.05% ('Ja') and 5.10 ± 0.10% ('Ou'). The crude protein values were 14.69 ± 0.00% ('Ou'), 10.30 ± 0.01% ('CF1'), 3.76 ± 0.05% ('Va') and 2.58 ± 0.03% ('Ja') and lipid content 1.39 ± 0.01% ('Va'), 1.38 ± 0.02% ('Ou'), 1.36 ± 0.05% ('CF1') and 1.09 ± 0.00% ('Ja'). Also, the results of dry matter were 68.41 ± 0.08 to 94.89 ± 0.01%, ashes 14.70 ± 0.01 to 33.26 ± 0.05%, fiber content 30.78 ±0.02 to 55.09 ± 0.00%, NFE 7.78 ± 0.19 to 49.27 ± 0.04% and gross energy 1,193.33 ± 1.15 to 4,417.00 ± 1.73 Kcal/kg. The best source of protein was 'Ou'', for lipids, NFE and gross energy was for 'Va', for minerals and fiber content was 'Ja'.

Table 2. Physicochemical analysis of mucilage's extracted from different nopal cultivars (45 days of maturity) grown in a hydroponic system

Mucilage powders				
Analysis	'CF1'	'Va'	'Ja'	'Ou'
pH	5.19 ± 0.00 ^{d}	5.58 ± 0.03 ^{a}	5.47 ± 0.04 ^{b}	5.39 ± 0.02 ^{c}
TSS (°Brix)	15.31 ± 0.35 ^{d}	16.88 ± 0.32 ^{b}	17.65 ± 0.38 ^{a}	16.06 ± 0.11 ^{c}
Colour L*	81.54 ± 0.46 ^{a}	72.85 ± 0.05 ^{b}	67.46 ± 0.03 ^{c}	65.20 ± 1.93 ^{d}
a*	-4.54 ± 0.05 ^{d}	0.54 ± 0.67 ^{a}	-2.29 ± 0.35 ^{b}	3.09 ± 0.19 ^{c}
b*	16.76 ± 1.00 ^{d}	23.31 ± 0.53 ^{a}	20.87 ± 0.53 ^{c}	20.53 ± 3.20 ^{bc}

Different letters indicate significant difference ($p \leq 0.05$) between the cultivars (n = 3).

Table 3. Proximal composition of the mucilage extracted from different nopal cultivars (45 days of maturity) grown in a hydroponic system

Component (%)	'CF1'	'Va'	'Ja'	'Ou'
Moisture content	10.52 ± 0.09^{b}	31.26 ± 0.25^{a}	6.05 ± 0.05^{c}	5.10 ± 0.10^{d}
Dry matter	89.39 ± 0.01^{c}	68.41 ± 0.08^{c}	94.00 ± 0.10^{b}	94.89 ± 0.01^{a}
Protein	10.30 ± 0.01^{b}	3.76 ± 0.05^{c}	2.58 ± 0.03^{d}	14.69 ± 0.00^{a}
Lipid	1.36 ± 0.05^{b}	1.39 ± 0.01^{a}	1.09 ± 0.00^{c}	1.38 ± 0.02^{ab}
Ash	19.69 ± 0.01^{b}	14.70 ± 0.01^{c}	33.26 ± 0.05^{a}	16.89 ± 0.005^{d}
Fiber content	37.19 ± 0.01^{c}	30.78 ± 0.02^{d}	55.09 ± 0.00^{a}	39.28 ± 0.02^{b}
NFE	31.19 ± 0.01^{b}	49.27 ± 0.04^{a}	7.78 ± 0.19^{d}	27.63 ± 0.11^{c}
*Gross energy	3,373.66 ± 0.57^{c}	4,417.00 ± 1.73^{a}	1,193.33 ± 1.15^{d}	3,612.50 ± 0.86^{b}

Different letters indicate significant difference ($p \leq 0.05$) between the cultivars (n = 3).

Total polyphenols content (TPC)

The quantification of TPC can be an indication of the bioactivity and/or functionality of the crop and the TPC in the cultivars evaluated in this work was high that found by other authors. TPC varied from 565.76 ± 1.5 to 1,311.83 ± 2.3 mg GAE g^{-1} of dried weight (Table 4). Among the four cultivars studied, significant differences ($p \leq 0.05$) were found. The low values were found in 'Ja' (565.76 ± 1.5 mg GAE g^{-1}) and 'CF1' (593.81 ± 2.8 mg GAE g^{-1}), whereas 'Va' contained the highest amount (1,311.83 ± 2.3 mg GAE g^{-1}).

Antioxidant activity

DPPH-free radicals-scavenging assay is often used to determine the antioxidant potential of natural compounds. A lower absorbance demonstrates higher DPPH-free radicals-scavenging potential (Keshani-Dokht *et al.*, 2018) and one antioxidant have the potential to inhibit the formation of green-blue ABTS radicals because this test is based on the transfer of electrons and hydrogen atoms. It can be used to evaluate antioxidant activities of both hydrophilic and lipophilic compounds (Akbarbaglu *et al.*, 2019). Ferric reducing power assay is an indicator of the ability of bioactive compounds to donate an electron. In this assay, the conversion of the ferric cyanide complex (Fe^{3+}) to its reduced form (Fe^{2+}) is caused by the presence of antioxidants in the sample. The values for antioxidant activity shown in Table 4. For $DPPH^{\bullet}$ was 196.86. 86 ±1.52 mg GAE g^{-1} ('Ja'), 199.58 ± 0.23 mg GAE g^{-1} ('CF1'), 358.26 ± 0.53 mg GAE g^{-1} ('Va') and 739.54 ± 0.32 mg GAE g^{-1} ('Ou'). For $ABTS^{\bullet +}$ was 2,242.95 ± 0.99 mg TE g^{-1} ('CF1'), 3,140.05 ± 0.10 mg TE g^{-1} ('Ou'), 4,875.04 ± 0.72 mg TE g^{-1} ('Ja') and 6,301.12 ± 0.61 mg TE g^{-1} ('Va'). For FRAP was 125.79 ± 0.42 mg GAE g^{-1} ('Ja'), 181.02 ± 0.92 mg GAE g^{-1} ('Ou'), 216.38 ± 0.47 mg GAE g^{-1} ('CF1') and 536.26 ± 0.84 mg GAE g^{-1} ('Va'). The mucilage with the most antioxidant capacity was 'Va' and is consistent with the biggest concentration of TPC (1,311.83 mg GAE g^{-1}).

Table 4. Total polyphenols and antioxidant activity by B) method ABTS, C) FRAP and D) DPPH, of mucilage's extracted from various nopal cultivars grown in a hydroponic system

Mucilage's	TPC (mg GAE g^{-1})	DPPH• (mg GAE g^{-1})	ABTS•+ (mg TE g^{-1})	FRAP (mg GAE g^{-1})
'CF1'	593.81 ± 2.85^{d}	199.58 ± 0.23^{d}	2,242.95 ± 0.99^{b}	216.38 ± 0.47^{d}
'Va'	1,311.83 ± 2.36^{c}	358.26 ± 0.53^{c}	6,301.12 ± 0.61^{b}	536.26 ± 0.84^{b}
'Ja'	565.76 ± 1.50^{a}	196.86 ± 1.52^{b}	4,875.04 ± 0.72^{a}	125.79 ± 0.42^{a}
'Ou'	687.14 ± 1.17^{b}	739.54 ± 0.32^{a}	3,140.05 ± 0.10^{c}	181.02 ± 0.92^{c}

Different letters indicate significant difference (p≤0.05) between the cultivars (n =3).

Crystallinity and chemical bonding constituents

Cultivars 'Va' and 'CF1' were selected to perform FT-IR and X-ray diffraction considering that they had the best physicochemical properties (**Figure 2**). The first band which ranged between 3400 and 300 cm^{-1} represents the -OH region (Cai *et al.*, 2008; Contreras-Padilla *et al.*, 2016; Mejías Díaz *et al.*, 2013; Wang *et al.*, 2007). The second and third bands, ranging between 3000-2800 cm^{-1}, are representative of the -CH, $-CH_2$ and $-CH_3$ groups. In the bands 1500 cm^{-1}, the C = C group with low intensity is visible, which coincides with aromatic and alkene vibrations (Rubinson and Rubinson, 2000). The frequencies of the bands > 1000 cm^{-1} were identified as the HCH- flexed bonds that are isometrically present, possibly at -CHO. Band 8 that oscillates in mucilage's > 500 cm^{-1} is associated with β-D-glucose. The last FT-IR spectrum of bands 9-10 was attributed to the N-H bond and -OH respectively (Monrroy *et al.*, 2017; Smith, 1998; Zhao *et al.*, 2017). The X-ray diffraction patterns of hydroponic mucilage with and without fiber is shown in **Figure 3**. In the first found was gypsum (with confirmation on peak four) and $CaCO_3$ in the second peak. The silicon (third peak) and the fourth peak, the calcium sulphate. Followed by potassium oxalate monohydrate and the last peak showed the calcium hydride in the mucilage's with fiber, but the same compound was absent in the mucilage's without fiber.

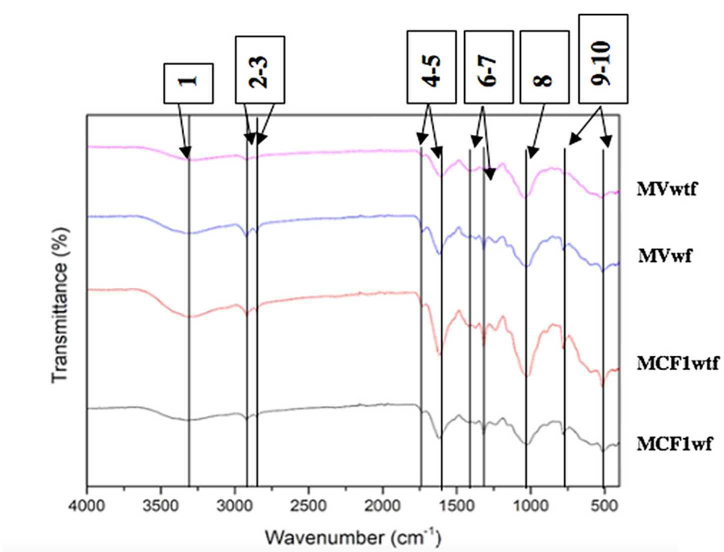

Figure 2. FT-IR spectra of 1) 'Villanueva' without fiber (MVwtf), 2) 'Villanueva' with fiber (MVwf), 3) 'Copena F1' without fiber (MCF1wtf) and 4) 'Copena F1' with fiber (MCF1wf) mucilage's

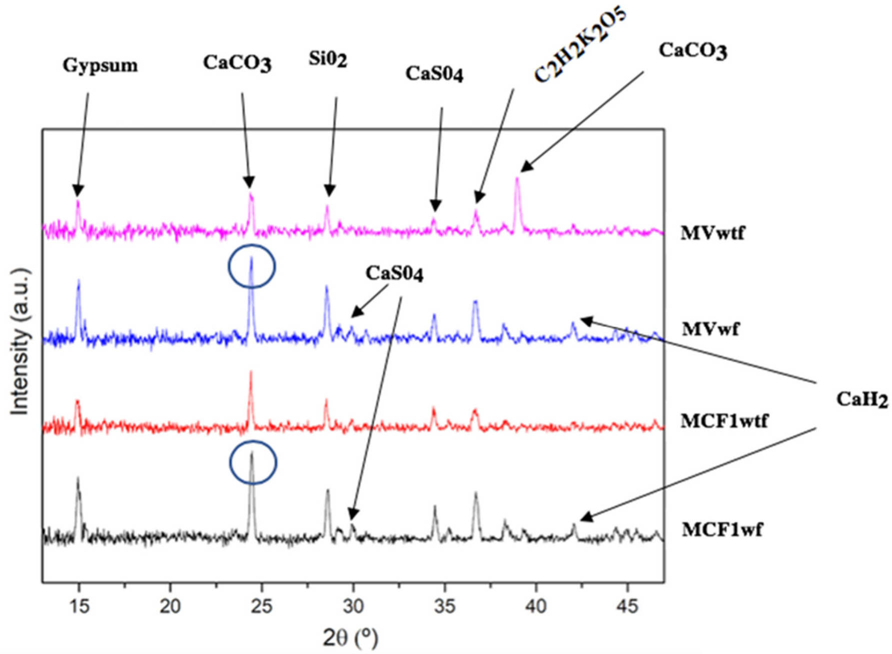

Figure 2. Incinerated diffraction patterns of the hydroponic mucilage's of cactus with 45 days of maturation

The mucilage's 'Villanueva' without fiber (MVwtf), 'Villanueva' with fiber (MVwf), 'Copena F1' without fiber (MCF1wtf) and 'Copena F1' with fiber (MCF1wf)

Discussion

Crop and yield

Other authors obtained 1,624 g m^{-2} of DB for 'Va' (21 cuts, 30 days and population density of 16 plants m^{-2}) in a closed hydroponic system (Almaguer-Sierra *et al.*, 2014) and 1,403.07 and 1,290.81 g m^{-2} (18 cuts, 15 days and population density of 14 plants m^{-2}) in open field (Flores-Mendiola, 2012). The use of hydroponic systems is better than traditional or intensive systems due to the use of water with high salt contents that produce highest values of yield, length and diameter with the cultivar 'Va', and with the 'CF1', and 'Ja', less (Ramírez-Tobías, 2012; Vázquez-Alvarado *et al.*, 2009). The difference in biomass production is attributed to the lack of assimilation of nutrients by some cultivars due to the competition for prickly pear production, given that, in shorter period, higher production of cladodes is obtained and the density of population to which the vegetative material established produces more (Rodríguez-Fuentes *et al.*, 2011). Also, air temperature is one factor since these conditions prevail in their maximum production during the spring-summer period. The traditional systems produce up to 90 tons ha^{-1} yr^{-1} and hydroponic systems up to 470 tons ha^{-1} yr^{-1}. This variability is due to the management systems, the differential genetic potential of the variety, que quality and the quantity of the climatic supply of different state. On the other hand, the optimal nutrients supply according to the cultivar and its phenological stage (Horibe, 2018).

Mucilage yields from 3.8 to 19% has been reported (Matsuhiro *et al.*, 2006; Sepúlveda *et al.*, 2007; Cai *et al.*, 2008; Contreras-Padilla *et al.*, 2016). The yield depends of cultivar type, crop age and vary with the extraction conditions, as well as environment and genotype (Kaur *et al.*, 2018). Also, the temperature (up to 110 °C) interfere with the yield due to the polysaccharides begin to degrade al 90 °C (Huang *et al.*, 2000; Karazhiyan *et al.*, 2011) and the use of alkaline conditions could increase the yield by hydrolyzing soluble and insoluble components (Hong and Ibrahim, 2013). However, the use of controlled conditions (hydroponic culture) allows to increase the concentration of mucilage because it reaches the physiological maturity of the culture in a fast and controlled way (Hooshmand *et al.*, 2019).

Physicochemical and proximal analysis

The obtained data on the pH of mucilage from *Opuntia ficus-indica* are scare in the available literature. However, similar values have been reported from 5.5 to 6.0 (León-Martínez *et al.*, 2011; Contreras-Padilla *et al.*, 2016; Monroy-Gutiérrez *et al.*, 2017). The difference is attributed to the accumulation of citric and malic acid in the cladodes before extracting the polymer, due to the presence of Ca_2 (due to the hydroponic culture system) increased the pH (Du Toit *et al.*, 2019; Trachtenberg and Mayer, 1980) and the regeneration of carbohydrates and accumulation of acids or different precursors of the enzyme phosphoenol-pyruvate could generate a positive increase in the acidity of the mucilage's at the time of being extracted. These values obtained for TSS are higher than that reported on literature and is due to the specie, hydroponic culture system and stage of maturity of the nopal (Madera-Santana *et al.*, 2018), which allows adequate physiological development due to the ease of nutrient uptake. The positive values of a* in the mucilage 'Va' and 'Ou' indicate a slight brown coloration that is attributed to alterations due to the heating process during the extraction of the mucilage. This caused a change of olive-green color to brown in the samples, due to the formation of pheophytin (Du Toit *et al.*, 2019). According to determination of color of the mucilage's, it is suggested that these might contain pigments like carotenoid, which means that they have an attractive antioxidant power for use in the industry (Hong and Ibrahim, 2013).

For moisture, several studies reported values of 3.44 to 8.27% (Rodríguez-González *et al.*, 2014), 7.09% (Treviño-Garza *et al.*, 2017) and 9.65% (Dick *et al.*, 2019). Our results are due to the storage of water retained by the solvent trawl for the recovery of the mucilage powder, the variety, quantity of water retention of polymers, matter stage and other factors.

These results are higher than reported in literature with moisture content up to 9.65% (Rodríguez-González *et al.*, 2014; Treviño-Garza *et al.*, 2017; Dick *et al.*, 2019), crude protein up to 19% (Lima Junior *et al.*, 2013; Martin *et al.*, 2017; Madera-Santana *et al.*, 2018; Dick *et al.*, 2019), total ashes up to 21% (Espino-Díaz *et al.*, 2010; Treviño-Garza *et al.*, 2017; Madera-Santana *et al.*, 2018; Du Toit *et al.*, 2019), crude fiber up to 55.33% (Dick *et al.*, 2019; Du Toit *et al.*, 2019), Nitrogen-free extract up to 1.28% (Madera-Santana *et al.*, 2018; Sepúlveda *et al.*, 2007). There is great variation on the protein content in the mucilage, however, this variation is due to the vegetative material and maturity (Martin *et al.*, 2017; Dick *et al.*, 2019). Likewise, this is due to the water retention in nopal during nutrition using the hydroponic system, variability of the genetic material and the mucilage's can acquire nitrogen from various sources, such as nucleic acids, nitrates and nitrites that were reserved by the nopal cactus (Madera-Santana *et al.*, 2018). Furthermore, the high values of ashes in the cladodes can be attributed to the high salinity of the soil and to the mineral bioavailability in hydroponic systems (Rodríguez-Fuentes, 2009). The mucilage's extracted from the nopal are highly rich in dietary fiber, this variation occurs at higher concentration as the nopal matures, since the fibers vary in their structure and size and therefore in the not solubilization or solubilization in the aqueous medium. Mucilage's have organic molecules that are considered hydrophobic and hydrophilic because their covalent bonds of hydrogen and carbon are not polarized, which distances them from polarized water molecules. This same property makes them insoluble in water or totally soluble (Rodríguez-Fuentes, 2009; Espino-Díaz *et al.*, 2010; Contreras-Padilla *et al.*, 2016). In general, these differences revealed the behavior of the mucilage cells present in the cactus cultivars and the accumulation of evaluated components, as the cladode matures, the nutritional content increases, since it reserves energy.

Total polyphenols content (TPC)

Reported values varied from 0.77 to 25.54 mg GAE g^{-1} (Jaramillo-Flores *et al.*, 2003; Kim *et al.*, 2013; Sangeethapriya and Siddhuraju, 2014). This variability is due to the generation of ascorbic acid present in the nopal mucilage's for the generation of phenolic compounds by providing hydrogen atoms to the phenoxy radicals, which are formed when the phenols are oxidized (Keshani-Dokht *et al.*, 2018). Besides, interaction of this vitamin with polyphenoloxidase causes irreversible inhibition of the tendency (Zheng and Wang, 2001; Wojdyło *et al.*, 2007). Additionally, it was observed that the concentrations studied depend on the state of the polymeric material, variety, stage of maturity and the hydroponic system allows the formation of phenolic compounds because the crop grows in optimal conditions compared to a traditional system (Mattson and Lieth, 2019; Xu *et al.*, 2019).

Antioxidant activity

Several reports inform about the antioxidant capacity of mucilage but not at these levels (Kim *et al.*, 2013; Motiwala *et al.*, 2015). This is due to the chemical composition of each species, the culture system and the concentration of the natural antioxidants that can have a synergist effect between the bioactive compounds that make up the mucilage for each cultivar. The differences between the results obtained between methods has already been described, as in the work of Floegel *et al.* (2011) whose research focused on comparing these methods and showed that the antioxidant capacity was better reflected by $ABTS^{\bullet+}$ than by $DPPH^{\bullet}$ in a wide variety of foods. The $DPPH^{\bullet}$ method is more selective because it does not react with flavonoids lacking hydroxyl groups in the B ring, nor with aromatic acids containing a single hydroxyl group.

Crystallinity and chemical bonding constituents

The hydroxyl group (3400 and 300 cm^{-1}) is characteristic of the accumulation of malic acid in the chlorenchyma and parenchyma, some of the most abundant acids and that are easily metabolizable by the microorganisms that attack the cactus. In addition, the -OH group is generated by chemical synthesis for the extraction of mucilage through precipitation (band 1). The presence of -CH, CH_2 and $-CH_3$ groups is explained by the carboxyl and xylane groups stored in lignocellulic layers of the mucilage before being recovered (Contreras-Padilla *et al.*, 2016; Monrroy *et al.*, 2017). The C = C, HCH- and -COH possibly by the formation of arabinose and dispersed carbohydrate groups such as mannose and glucose (Cai *et al.*, 2008; Mejías Díaz *et al.*, 2013; Monrroy *et al.*, 2017; Madera-Santana *et al.*, 2018). The results indicated that the maturation and the hydroponic culture system of the mucilage's does not significantly infer the intensity of the bands of the functional groups.

The X-ray diffraction patterns of hydroponic mucilage with and without fiber had the longest peaks that could be due to the larger particle size that the lens could inhibit the perception of crystallized compounds (Contreras-Padilla *et al.*, 2016; Marin *et al.*, 2018). The variation is due to the type of cultivar and culture system where the polymers were extracted, the maturity and the fiber accumulation in these. Calcium is available in the cactus nopal unless they have high concentrations of oxalic acid or dietary fiber since they limit their formation when they are sequestered as oxalates, preventing their absorption by the metabolism (Valenzuela *et al.*, 2018). The silicon (third peak) was absorbed in the mucilage's formed by nopal through nutrition in the form of monosilicic acid by the assimilation of nutrients captured during the hydroponic crop cycle. In the fourth peak, the calcium sulphate that has been reported in the structure of parenchyma nopal, calcium oxalate crystals are formed. These are classified into four types by their morphology: rafidia, drusen, styloid and prisms (Bárcenas *et al.*, 2011; Madera-Santana *et al.*, 2018). Hydrogen oxalate hydrate of potassium is considered an antinutrient that is in the form of calcium crystals or free form and change depending on the maturity of the mucilage (Nerkar and Gattani, 2012; Contreras-Padilla *et al.*, 2016). The calcium carbonate in the fibrous mucilage's can occur in three anhydrous crystalline polymorphs such as calcite, aragonite and vaterite ordered by their thermodynamic stability (Payne *et al.*, 2007). The last peak showed the calcium hydride in the mucilage's with fiber, but the same compound was absent in the mucilage's without fiber. This could be due to the expression of oxalates crystallized directly in the fibrillar tubes of the nopal tissues where the mucilage is stored (Trachtenberg and Mayer, 1982).

Conclusions

A hydroponic system to produce mucilage is more efficient in quantity and quality. However, 'Copena F1' at culture conditions of 30 plants m^{-2} is the best alternative to produce biomass and mucilage. 'Villanueva' allows to obtain higher concentrations of TPC (1,311.83 ± 2.36mg GAE g^{-1}) with a high antioxidant capacity $ABTS^{\bullet+}$ (6,301.12 ± 0.61 mg TE g^{-1}), FRAP (536.26 ± 0.84 mg GAE g^{-1}) and $DPPH^{\bullet}$ (358.26 ± 0.53 mg GAE g^{-1}) with a large amount of lipids (1.39 ± 0.01%), nitrogen-free extract (49.27 ± 0.04%) and energy content (4,417.00 ± 1.73 Kcal/kg). The identification of the characteristic functional groups of the mucilage (-OH, -CH, $-CH_2$, $-CH_3$, C=C, HCH, -CHO) and gypsum, cellulose, SiO_2 $CaSO_4$, $C_2H_2K_2O_5$, $CaCO_3$ and CaH_2 by X-ray diffraction was also done. This study provides a novel, cost-effective and applicable technology in any region without the need for large tracts of land to produce high quality cactus and mucilage with bioactive properties.

Authors' Contributions

Investigation, resources, and writing – original draft preparation: B.L.-S.; supervision: H.R.-F., M.A.C. and L.M.P.; writing – reviewing and editing: G.C.G.M.-Á.; methodology: A.G.A. and D.C.G.-S.; funding acquisition and project administration: R.R. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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