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Research Article

Functional properties and bioactive compounds of *Opuntia* mucilage under different irrigation regimes and its use as an additive in an edible coating

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Abstract

Nopal (*Opuntia*) varieties 'Amarilla Olorosa' 'Cristalina', 'Dalia Roja' and 'Roja Lisa' were subjected to three irrigation treatments: without irrigation (WI), supplementary irrigation (SI) (field capacity 0.28 m^3 m^{-3} and 0.14 m^3 m^{-3} permanent wilt point), and complete irrigation (CI) (100 % of crop evapotranspiration) for ~4 months. Mucilage was extracted from harvested cladodes for evaluation of swelling, water retention capacity (WRC), solubility, oil retention capacity (ORC), total polyphenol content (TPC), polyphenol profile, and antioxidant capacity. The highest swelling rates were found in the mucilage of 'Roja Lisa' and 'Crystalline' WI. The mucilage of 'Roja Lisa' WI presented the highest WRC and solubility. The highest ORC values were exhibited by the mucilage from 'Amarillo Olorosa' and 'Dalia Roja' CI. Likewise, the mucilage of 'Cristalina' SI showed the highest TPC and antioxidant capacity. Phloretin, pterostilbene, and sinensetin were identified in the mucilage of the four varieties of nopal. Also, edible films of sodium alginate, fortified with Cristalina WI mucilage, were made. To improve the mechanical properties and nutritional composition of the coatings, edible films of sodium alginate were produced and enriched with Cristalina WI mucilage. The coatings presented low opacity and permeability to water vapor. Thus, limiting water to cactus plants could be a viable strategy to produce functional and bioactive mucilage that can be used as edible coating material for fruits and vegetables.

Keywords: Antioxidants; cladodes; film; polyphenolic compounds; technological properties

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Introduction

The cacti belonging to the genus *Opuntia*, known in Mexico as nopal, are plants endemic to arid and semi-arid areas that have a minimal water requirement for growth (Jorge *et al.,* 2023). In certain regions, young cladodes (approximately 6 months of growth) are consumed as a food source for humans. In addition to serving as food, nopal intake is known to provide essential health benefits; it can prevent diabetes (Gouws *et al.,* 2022), hypercholesterolemia (Gouws *et al.,* 2020), obesity (Sirotkin, 2022), and hypertension (Pandit *et al.,* 2020), among others. These benefits have been associated with the presence of secondary metabolites, mainly phenolic compounds, in the cactus (Câmara *et al.,* 2021; Hernandez-Becerra *et al.,* 2022). Older cactus cladodes (medium to advanced maturity) are not used in the human diet due to the undesirable textural properties that come from high content of insoluble fiber (Contreras-Padilla *et al.,* 2016). These older cladodes must be pruned annually, which produces 10-15 t ha^{-1} of leaf waste (Zegbe *et al.,* 2015). That plant material can be used as fodder (Hernandez-Becerra *et al.,* 2022)or applied to soil to counteract its erosion in arid areas (Malainine *et al.,* 2003). Since a large amount of this plant material is disposed of, finding other uses for it in different industrial sectors is crucial. One of the products of great value that can be obtained from the nopal is its mucilage. It is known that this extract can be used as an ingredient in the food, agronomic, textile, chemical, and pharmaceutical industries, among others (Guevara-Arauza, 2021).

The mucilage of the prickly pear cactus, nopal, is mainly composed of hydrocolloidal heteropolysaccharides (Caldera-Villalobos *et al.,* 2022), which have varying proportions of arabinose, galactose, rhamnose, and xylose (Morales-Chávez *et al.,* 2024). Additionally, it contains proteins, minerals, lipids, and uronic acids (Goksen *et al.,* 2023). Cactus mucilage has promising prospects as an additive due to its foaming and emulsifying properties, water and oil retention, viscosity modification, and excellent antioxidant activity (Bayar *et al.,* 2016). Currently, mucilage has been widely utilized as a thickening agent (Carpintero-Tepole *et al*., 2021), a fat substitute (Bernardino-Nicanor *et al.,* 2015), a stabilizer (Du Toit *et al.,* 2019), and a material for encapsulating bioactive compounds (Soto-Castro *et al.,* 2019).

However, the mucilage content, its chemical composition, and functional properties are strongly influenced by various factors, such as genotype, climatic conditions, plant age, harvest time, and soil water content (Du Toit *et al.,* 2020; Pinheiro *et al.,* 2024). During dry periods, plants accumulate carbohydrates to facilitate water movement within their tissues and enhance their survival. Also, polyphenolic compound content is higher in cladodes collected at night (Sáenz *et al.,* 2004; Pinheiro *et al.,* 2024). Therefore, it is of utmost importance to consider these factors to maintain the constant nutritional and functional quality of mucilage. At the same time, it is well known that drought-stressed nopal plants produce fewer cladodes. Therefore, to increase the yield and production of cladodes in cactus plants without affecting the nutritional and functional properties of the plant components, various agronomic practices such as supplementary irrigation (SI) have been implemented (Zegbe and Servin-Palestina, 2020).

Supplemental irrigation is an addition of a limited water amount (~30, 50, or 70% of the amount of complete irrigation) to rain-fed crops when rain does not provide the essential moisture for normal plant growth to improve and stabilize productivity (Oweis and Hachum, 2005; Ding *et al.,* 2021). This method helps to keep soil moisture at a sufficient (high, but not excessive) level and eliminates water deficit in the plants' root zone (Satognon *et al.,* 2021). Likewise, SI allows better control of the planting date, resulting in better growth, higher biomass production, and favorable environmental conditions (Oweis and Hachum, 2005). For example, it has been shown that the application of SI increases the output of nopal biomass up to two times (Neupane *et al.,* 2021) and increases the production and quality of the nopal fruit (Zegbe *et al.,* 2015; Arba *et al.,* 2018; Zegbe *et al.,* 2020). It has also been documented that SI can increase wheat yield without having a relevant nutritional impact on antioxidant and mineral micronutrient contents (Wang *et al.,* 2021). Likewise, the antioxidant capacity of hazelnuts increases with the SI application (Tonkaz *et al.,* 2020).

However, the effect of supplying different amounts of water to *Opuntia* plants on the yield, functional properties, and antioxidant capacity of the mucilage is unknown. Therefore, this study aimed to analyze the changes in the functional properties, total phenolic content, and antioxidant capacity of mucilage extracted from cladodes of four nopal varieties subjected to different irrigation regimes. Moreover, edible films based on sodium alginate fortified with the mucilage obtained were elaborated.

Materials and Methods

Chemical reagents

Sodium alginate, Tween 80, and soy lecithin were supplied by Golden Bell (México, D.F.). Acetic acid, calcium chloride, sorbitol, sodium hydroxide glycerol, monobasic sodium phosphate, Folin-Ciocalteu reagent, gallic acid, ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), potassium persulfate, Trolox, TPTZ (2,4,6-Tris(2-pyridyl)-striazine), HPLC grade solvents (formic acid, water, acetonitrile, and methanol) were acquired from Sigma-Aldrich (Louis MI, USA). Sodium carbonate, 96% ethanol, and distilled water came from Analytyka® S.A de CV (Nuevo León, México).

Vegetal material

13-year-old prickly pear cactus of 'Amarilla Olorosa' varieties (*Opuntia* spp.), 'Cristalina' (*Opuntia albicarpa* Scheinvar), 'Dalia Roja' (*Opuntia* spp.), and 'Roja Lisa' (*O. ficus-indica* (L.) Mill), were used in this study. The samples were obtained from the National Institute of Forestry, Agricultural, and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP), Zacatecas Calera Experimental Field, Mexico (latitude 22°54'N, longitude 102°39'W, elevation 2.197 m).

Experimental process

Nopal varieties 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja', and 'Roja Lisa' were subjected to different irrigation conditions: without irrigation (WI) as a control, supplementary irrigation (SI), and complete irrigation (CI). Under WI, plants received only rainwater (91 mm); those under SI received irrigation at field capacity (FC = 0.28 $m^3 m^{-3}$), that is, when the water content in the soil (θ) was close to the point of permanent wilting (PWP = 0.14 $m^3 m^{-3}$); while plants under CI received weekly 100% of the evapotranspiration of the crop, which was estimated through a water balance. The experiment was conducted from February to May (the dry period) in 2019.

The experiment was conducted using a completely randomized plot design with three repetitions. The experimental unit consisted of 10 kg of reproductive cladodes (one-year-old), free from mechanical damage and pests, obtained from nine 13-year-old plants for each treatment.

Mucilage Extraction

The extraction process was carried out as indicated by Madera-Santana *et al.,* (2018). Cladodes of the cactus variety, subjected to the different types of irrigation, were manually deboned and sanitized with chlorinated water at 200 ppm. The epidermis was removed and cut into cubes (~1 cm^3). Subsequently, 100 g of cladode cubes and distilled water (in a 1:10 w/v ratio) were blended for 45 seconds. The pH of the mixture was adjusted to 7.00 ± 0.1 with 5 N NaOH, heated to 50 °C with constant agitation for 16 h, and filtered through a sieve. Subsequently, the filtrate was mixed with 96% ethanol (1:2 v/v). The mixture was shaken for 2 hours at room temperature and stored at 4 °C for 24 hours. The supernatant was then decanted, and the precipitate was concentrated in a rotary evaporator (BUCHI R-210, Switzerland) under vacuum at 50 °C, 175 mbar and 50 rpm. The concentrate was freeze-dried and vacuum-packed. The mucilage yield was ~14.4 5%.

The lyophilized mucilage was subjected to analysis of functional properties, total polyphenolic content, identification of polyphenolic compounds, and antioxidant capacity.

Functional properties of mucilage

Swelling

Samples of lyophilized mucilage (0.15 g) were mixed with 5 mL of sodium phosphate buffer (1 M, pH 6), stirred in a vortex for 1 min, and their initial volume was recorded. The suspensions were shaken and allowed to rest for 16 h at room temperature, and the final volume was noted (Deeksha *et al.,* 2014). The swelling index was measured with the following equation:

$$Swelling\ index\ (\%) = \frac{Final\ Volume - Initial\ Volume}{Final\ Volume} \tag{1}$$

Water retention capacity

Water retention capacity (WRC) was determined according to the method proposed by Femenia *et al.,* (1997). Exactly 0.15 g of freeze-dried mucilage was stirred in 5 mL of sodium phosphate buffer (1 M, pH 6) using a vortex for 1 min and then allowed to stand for 24 h at room temperature. Then, the mixture was centrifuged at 3800 g for 15 min. The residual solids of the supernatant were recovered by filtration (filter paper GF/C) and recombined with the sediment. The sediment was weighed (P1) and dried at 100 ± 2 °C overnight. After cooling, the dry weight (P2) was determined, and the WRC was calculated using the equation. 2:

$$WRC = \frac{P_1 - P_2}{P_2 - k} \tag{2}$$

Where k = α (P1−P2) with α = 0.028 g phosphate /mL.

WRC units were expressed as g H_2O/g mucilage.

Oil retention capacity (ORC)

Mucilage samples (0.15 g) were mixed with 5 mL of sunflower oil and stirred in a vortex for 1 min. The suspensions were left to stand for 12 h at room temperature and then centrifuged at 3800 g for 10 min. The supernatant was removed, and the solids were weighed. ORC was expressed as g oil/g mucilage (Minjares-Fuentes *et al.,* 2017).

Solubility

The solubility test was carried out according to the method proposed by Dick *et al.,* (2019) with some modifications. 0.25 g of freeze-dried mucilage was weighed and added to 10 mL of distilled water at 60 °C. The mixture was stirred in a vortex and homogenized using an Ultraturrax IKA T25D (IKA Works, Inc., Wilmington, USA) at 13,500 rpm for 1 min. The samples were subjected to magnetic stirring at 200 RPM for 2 hours at 60 °C to complete their hydration. The solutions were then centrifuged at 3800 g for 20 minutes, and the supernatant was filtered through a fiberglass (GF/C) filter. The precipitate was oven-dried at 100 °C for 24 h and weighed. The solubility percentage was calculated using the following equation:

$$solubility\ \% = \frac{P_1 - P_2}{P_1} * 100 \tag{3}$$

Where P_1 is the initial weight of the sample, and P_2 is the dry weight of the precipitate.

Total polyphenol content

Total polyphenols. Solutions of 10 mg of freeze-dried mucilage in 1 mL of water were used for the assay. The measurement was carried out using the Folin-Ciocalteu method, as described by González-Centeno *et al.,* (2012). Exactly 100 µL of mucilage solution, 950 µL of distilled water, and 50 µL of Folin-Ciocalteau reagent were mixed and vortexed. After 5 min, 800 µL of Na_2CO_3 (7.5% w/v) was added and vortexed. The mixture was incubated for 1 h in the dark at 25 °C. The absorbance of the solution was read at 765 nm in a UV-Vis spectrophotometer (HACH DR 5000, Mexico). The phenolic content was calculated using a standard curve with gallic acid as the standard ($R^2 = 0.99$); the results were reported in mg of gallic acid equivalent per g of dry weight (DW). The analyses were performed in triplicate

Identification of polyphenolic compounds by HPLC/ESI/MS analysis

Liquid chromatography analysis was performed on a Varian system with a PDA detector (ProStar 330, Varian, Atlanta, GA, USA) and an ion-trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, Palo Alto, CA, USA). Approximately 10 mg of freeze-dried mucilage was mixed with 1 mL of water using a vortex stirrer; the resulting solution was then filtered through a 0.45 µm PTFE filter. The injection volume was 5 µL. The column Denali C18 (150 mm × 2.1 mm, 3 µm, Grace, Albany, OR, USA) was kept at 30 °C and used for analysis. Formic acid (0.2%, v/v; A) and acetonitrile (B) were used as the mobile phase at a flow rate of 0.2 mL/min. The gradient used was: 3% B; 0-5 min, 9% B linear; 5-15 min, 16% B linear; 15-45 min, 50% B linear. The PDA detector was programmed at 245, 280, 320, and 550 nm. MS assays were conducted in the negative mode [M-H]- with nitrogen as a nebulizer gas and helium as a buffer gas. The ion source was maintained at 5.0 kV; the voltage and temperature of the capillary were 90 V and 350 °C, respectively. Data were collected and processed using MS Workstation software (v. 6.9). The scan mode was set to the m/z range of 50-2000. MS/MS analyses were performed on several selected precursor ions. Finally, the compounds were compared using a database of bioactive compounds (WorkStation database version 2.0, VARIAN, Palo Alto, CA, EE. UU.) (Hernández-Hernández *et al.,* 2020).

Antioxidant capacity

To determine antioxidant capacity using the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay, the methodology described by González-Centeno *et al.,* (2012) was used, with some modifications. ABTS (7 mmol L^{-1}) was mixed with potassium persulfate (2.45 mmol L^{-1}) and kept for 16 h at room temperature in the dark. At the time of analysis, 5 mL of the ABTS solution was diluted with distilled water in a 200 mL volumetric flask to obtain an absorbance of 0.700 ± 0.02 at 734 nm. To determine antioxidant capacity, 50 µL of the mucilage extract (10 mg mucilage in 2 mL distilled water) and 1.95 mL of ABTS solution were mixed. The mixture was incubated for 1 h at 25 °C, and then the absorbance of the sample was read at 734 nm in a UV-Vis spectrophotometer (HACH DR 5000, Mexico). Trolox standard solutions were prepared at a concentration of 0.2 to 1.2 mM, using water as the solvent. The results were expressed in µM equivalent in trolox/g DW.

To determine antioxidant capacity using the Ferric Reducing Antioxidant Power (FRAP) method, 130 µL of the extract (10 mg mucilage in 1 mL of distilled water) was mixed with 290 µL of 0.2 M phosphate buffer (pH 6.6) and 290 µL of 1% potassium ferricyanide (w/v). The samples were then incubated at 50 °C for 30 min. After incubation, 290 µL of 10% TCA (w/v), 1 mL of distilled water, and 250 µL of 0.1% ferric chloride (w/v) were added. After 30 min of incubation at 25 °C, the absorbance was measured at 700 nm in a UV-Vis spectrophotometer (HACH DR 5000, Mexico). The Trolox calibration curve was obtained using concentrations ranging from 0.2 to 1.2 mM. Antioxidant capacity was expressed in µM Trolox equivalent/g DW (Bayar *et al.,* 2016).

Technological use of mucilage

Extracts and polymers derived from plants have been utilized to create edible coatings. Mucilage extracted from the investigated varieties at different irrigation levels with the highest antioxidant potential and polyphenols (‘Crystalline’ WI) was selected to improve edible films.

Coating Preparation

The coating was developed according to the method proposed by Reyes-Avalos *et al.,* (2016). Approximately 1 g of mucilage and 1.25 g of sodium alginate were dissolved in 100 mL of distilled water at 55-60 °C. Subsequently, 600 mg of sorbitol, 5 mL of glycerol, 1.0 mL of Tween 80, 0.75 mL of soy lecithin, and 12 mL of olive oil were added. The mixture was homogenized at 22,000 rpm for 13 minutes using an Ultraturrax T18 (IKA Works, Inc., Wilmington, USA). The sodium alginate emulsion was then spread on smooth glass plates and immersed in a 2% calcium chloride solution (w/v) for 3 minutes. The film obtained was left to dry at room temperature (25 °C) for 12 h. The film thickness was measured using an optical micrometer (Labomed VF10X, Labomed Inc., CA, USA).

Coating Characterization

Opacity

The opacity of the films was determined according to the methodology proposed by Kanatt and Makwana (2020). The opacity was calculated using the following formula:

$$Opacity = \frac{600\,nm}{t} \tag{4}$$

Where t is the thickness of the film in mm (~0.088 ± 0.005 mm).

Water vapour permeability and mechanical properties

The film was placed on a beaker (50 mL) with 20 mL of distilled water. The weight and the percentage of relative humidity were recorded each hour for 12 h. The WVP values were calculated using the following equation:

$$WVP = \frac{WVRT}{[S(R_1 - R_2)]} D \tag{5}$$

Where WVTR is the water vapor transport rate ($g{\cdot}s^{-1}{\cdot}m^{-2}$); *S* is the vapor pressure of water (Pa) at 25 °C; R_1 y R_2 are the external and internal relative humidity (100 %), respectively; and *D* is the thickness of the film (m). For this, the WVTR parameter was determined according to the following equation:

$$WVTR = \frac{\frac{dm}{dt}}{A} \tag{6}$$

Where $\frac{dm}{dt}$ is the water vapor transmission rate expressed in $g\text{-}s^{-1}$, and A is the film area (m^2). All determinations were performed in triplicate (Reyes-Avalos *et al.,* 2016).

The mechanical properties of the film were determined on 20 rectangular samples of 2 x 7 mm (0.088 mm thick); before analysis, the samples were conditioned at 80 % relative humidity and 25 °C for 48 h. The maximum tensile strength, the maximum percentage elongation at break (%) and the Young's modulus were measured using a TA-XT plus texture analyzer (Stable Micro Systems, London, England). The films were stretched using a speed of 0.5 mm s^{-1}. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of original length). The data was analyzed with the Exponent software version 5.1.1.0 (Reyes-Avalos *et al.,* 2016).

Statistical analysis

The data obtained were statistically analyzed using variance analysis, and the comparison of means was performed using Fisher's LSD at a significance level of 0.05. All calculations were performed in the STATISTICA 7.0 system (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

Functional properties of mucilage

Water retention capacity (WRC) and swelling

Figure 1 shows the mucilage's water retention capacity, WRC, of four varieties of prickly pear cactus subjected to different irrigation regimes (WI, SI, CI). In general, the mucilage of the 'Roja Lisa' variety presented the highest WRC (~5.4 g H_2O/g mucilage), followed by the 'Amarilla Olorosa' WI (~4.9 g H_2O/g mucilage). Interestingly, the water supply did not affect the WRC in the 'Roja Lisa' variety. On the other hand, the mucilage with the lowest WRC were those obtained from the 'Crystalina' and 'Dalia Roja' varieties, with CI values of ~3.1 and 3.5 g H_2O/g mucilage, respectively. Likewise, Figure 2 shows that the mucilage of the 'Roja Lista' and 'Crystalina' WI varieties exhibited the highest swelling values (~6.2%) ($p \leq 0.05$), whereas most varieties with CI had the lowest values. Additionally, it can be observed that the varieties 'Amarilla Olorosa' and 'Dalia Roja' were not sensitive to changes in the amount of water available, as they exhibited similar swelling values across all irrigation systems. WRC and swelling index are properties of fibers that are related to the chemical structure of the polysaccharides that compose them, and to factors such as porosity, particle size, ionic shape, pH, temperature, ionic strength, and type of ions, among others (Elleuch *et al.,* 2011). The higher WRC values and swelling index in plants under limited irrigation (WI and SI treatments) could be attributed to a higher molecular weight of their polysaccharides, as well as a greater content of carboxylic acids, proteins, and uronic acids found in mucilage subjected to water stress (Luna-Zapién *et al.,* 2023). These factors favor better hydration of the plant tissue. Interestingly, in our study, the WRA and swelling index showed a strong positive correlation ($R^2 = 0.985$).

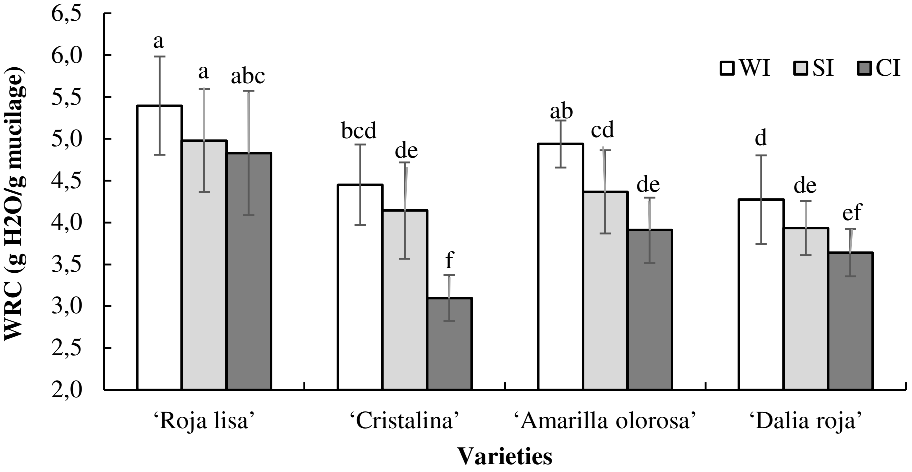

Figure 1. Water retention capacity (g H_2O/g mucilage) of freeze-dried mucilage of *Opuntia* spp. Varieties subjected to irrigation regimes: without irrigation (WI, control), supplementary irrigation (SI), and complete irrigation (CI)

Average values with different letters indicate statistical differences ($p \leq 0.05$) according to the LSD of the Fisher test. The vertical bars in each average indicate the standard deviation (n = 3)

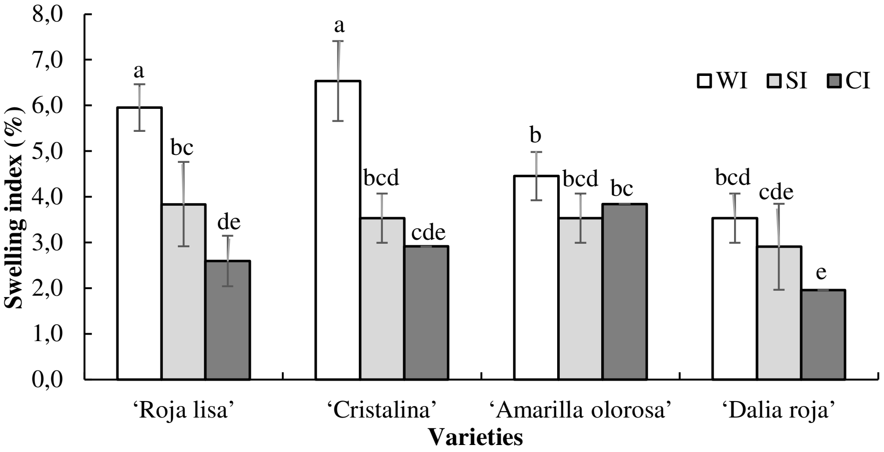

Figure 2. Swelling index (%) of freeze-dried mucilage of *Opuntia* spp. varieties subjected to irrigation regimes: without irrigation (WI, control), supplementary irrigation (SI), and complete irrigation (CI)
Average values with different letters indicate statistical differences ($p \leq 0.05$) according to the LSD of the Fisher test. The vertical bars in each average indicate the standard deviation ($n = 3$)

Solubility

Figure 3 illustrates the percentage solubility of mucilage in different varieties of nopal under three irrigation treatments. The 'Roja Lisa' mucilage exhibited the highest solubility when exposed to WI and SI treatments, at ~69% and 65%, respectively. Likewise, the mucilage of this variety presented the lowest solubility when CI was applied (58.2%). Similarly, the solubility of the mucilage of the varieties 'Cristalina', 'Amarilla Olorosa', and 'Dalia Roja' was not affected by the type of irrigation applied to the nopal. The solubility of polysaccharides is related to their structure and the presence of functional groups, such as COOH or SO_4^{-2} (Elleuch *et al.,* 2011). Thus, the increased presence of carboxyl groups and uronic acids in the mucilage compounds of the 'Roja Lisa' variety, subjected to WI and SI (Luna-Zapién *et al.,* 2023) produced more soluble material. The solubilization capacity of the prickly pear mucilage can improve the technological properties of liquid food products, which could help reduce the glycemic and triglyceride indexes in consumers (Cárdenas *et al.,* 2019).

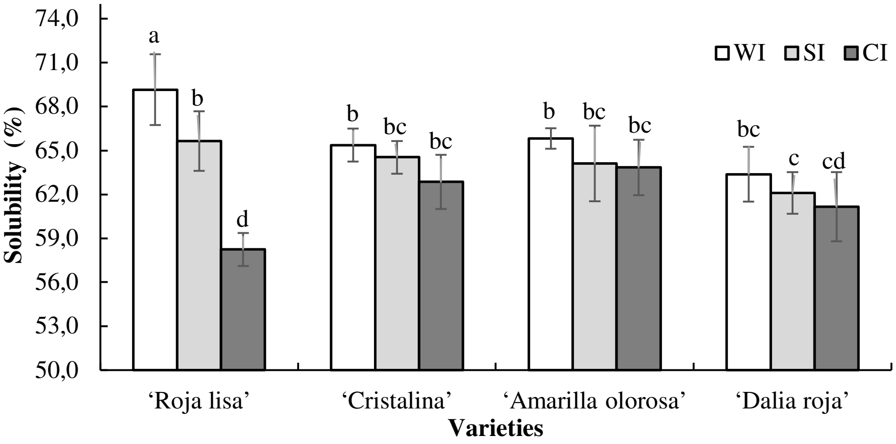

Figure 3. Solubility (%) of freeze-dried mucilage of *Opuntia* spp. varieties subjected to irrigation regimes: without irrigation (WI, control), supplementary irrigation (SI), and complete irrigation (CI)
Average values with different letters indicate statistical differences ($p \leq 0.05$) according to Fisher's least significant difference (LSD) test. The vertical bars in each average indicate the standard deviation ($n = 3$)

Oil retention capacity (ORC)

The ORC obtained from the mucilage of different nopal varieties exposed to WI, SI, and CI is shown in Figure 4. The varieties 'Amarilla Olorosa' and 'Dalia Roja' with CI, presented the highest values of this parameter ($p \leq 0.05$). Interestingly, the mucilage of the 'Red Dalia' variety was the most susceptible to changes in irrigation regime, generating the highest ORC (~9.3), with CI, and the lowest (~6.6%), with SI ($p \leq 0.05$). It is well established that the functional properties of the cell wall in plants depend on the composition and three-dimensional structure of the polysaccharides that comprise them (Jarvis, 2011). The scarcity or abundance of water influences the formation of fibers with varying amounts of polar and/or non-polar groups, making them more akin to hydrophilic or hydrophobic substances. Greater water availability in plants generates better non-polar zones in the mucilage and vice versa. Therefore, high mucilage WRC is correlated with lower mucilage ORC ($R^2 = 0.99$). The above findings align with those reported by Alvarado-Morales *et al.,* (2019), who noted that Aloe vera mucilage with high WRC presented lower ORC. Therefore, nopal mucilage with high ORC could be used as a stabilizer of high-fat products and/or emulsions.

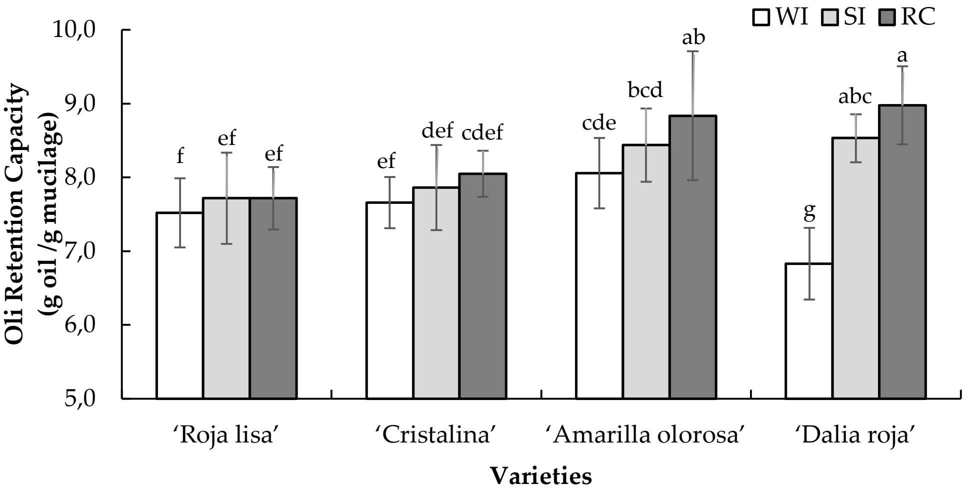

Figure 4. Oil Retention Capacity (g oil/g mucilage) of freeze-dried mucilage of varieties of *Opuntia* spp. subjected to irrigation regimes: without irrigation (WI, control), supplementary irrigation (SI), and complete irrigation (CI)

Average values with different letters indicate statistical differences ($p \leq 0.05$) according to the LSD of the Fisher test. The vertical bars in each average indicate the standard deviation ($n = 3$)

Total polyphenol content and identification of polyphenolic compounds in mucilage

The total polyphenol content (TPC), of the mucilage of the studied nopal varieties subjected to different types of irrigation ranged from 4.31 to 12.08 mg EAG/g dry weight (Table 1). The highest amount of TPC was found in the 'Cristalina' variety in the WI treatment (~12 EAG/g dry weight), followed by the same variety with SI (~10.2 EAG/g dry weight) and 'Amarilla Olorosa' WI (~10.7 EAG/g dry weight). The 'Roja Lisa' variety exhibited the lowest polyphenol values and was not affected by the amount of water supplied to the plant ($p > 0.05$). In contrast, in the remaining varieties studied, the type of irrigation affected the concentration of these compounds in the mucilage, with higher TPC observed under lower water availability treatment.

Table 1. Total polyphenol content and antioxidant capacity by ABTS and FRAP methods of *Opuntia* spp. mucilage subjected to irrigation regimes: No Irrigation (WI, control), Supplementary Irrigation (SI), and Complete Irrigation (CI)

Watering regime	Variety	TPC (mg EAG/g DW)	ABTS (µM eq. Trolox /g DW)	FRAP (µM eq. Trolox /g DW)
WI	'Roja Lisa'	4.89 ± 0.05 h	78.51 ± 0.82 g	15.68 ± 1.24 g
	'Cristalina'	12.08 ± 0.27 a	152.30 ± 3.29 a	58.28 ± 1.68 a
	'Amarilla Olorosa'	10.70 ± 0.64 b	137.96 ± 2.13 b	30.40 ± 0.53 c
	'Dalia Roja'	8.06 ± 0.27 f	126.54 ± 1.50 d	21.45 ± 0.20 e
SI	'Roja Lisa'	4.48 ± 0.14 hi	71.026 ± 2.92 h	12.74 ± 0.77 h
	'Cristalina'	10.20 ± 0.29 c	136.68 ± 5.24 bc	32.45 ± 2.17 b
	'Amarilla Olorosa'	9.72 ± 0.21 d	126.27 ± 1.65 d	24.99 ± 0.88 d
	'Dalia Roja'	6.82 ± 0.59 g	117.50 ± 1.35 e	19.77 ± 0.49 f
CI	'Roja Lisa'	4.31 ± 0.11 i	70.84 ± 2.17 h	12.07 ± 0.95 h
	'Cristalina'	9.55 ± 0.64 d	132.85 ± 2.12 c	24.08 ± 1.27 d
	'Amarilla Olorosa'	9.02 ± 0.33 e	120.06 ± 3.42 e	21.81 ± 1.05 e
	'Dalia Roja'	6.82 ± 0.09 g	104.90 ± 4.52 f	18.39 ± 0.31 f

Within each column, mean values (± standard deviation; n=3) with different letters indicate statistical differences ($p \leq 0.05$) according to the minimum significant difference of the Fisher test. DW = dry weight

The genetic variability and biochemical characteristics of each *Opuntia* species influence the amount of polyphenols (Alves *et al.,* 2017). Additionally, the increase in polyphenols can be attributed to the water stress to which the plants were subjected. Boutakiout *et al.,* (2018) observed a similar behavior in *Opuntia* cladodes, finding higher polyphenolic content and antioxidant capacity in cladodes of plants harvested during a drought period. The periods of extreme drought increase the production of secondary metabolites as a defense mechanism to water stress conditions (Boutakiout *et al.,* 2018).

The HPLC/ESI/MS analysis showed that the mucilage of the four varieties of prickly pear cactus investigated mainly contained phloretin, sinensetin, and pterostilbene; the latter stands out for its greater abundance (see supplementary material). Furthermore, the abundance of these compounds was similar in the cactus varieties investigated. Previously, these compounds have been found in extracts of *Opuntia ficus-indica* (Coronado-Contreras *et al.,* 2023). To our knowledge, this is the first report of these compounds in nopal mucilage. Interestingly, pterostilbene is considered an analogue of resveratrol, exhibiting anti-inflammatory and antioxidant properties (An *et al.,* 2022). Decreased liver fat and weight reduction, as well as reduction of inflammatory biomarkers and blood glucose, are attributed to its presence in the diet (Kim *et al.,* 2020). Phloretin and sinenestin have also been shown to possess biological activities such as anticancer, antioxidant, and anti-inflammatory effects (Yang *et al.,* 2022). The presence of these bioactive compounds in the mucilage of nopal makes it an attractive ingredient for use in the food and pharmaceutical industries.

Antioxidant capacity of mucilage

The antioxidant capacity (AC) of the mucilage of four varieties of *Opuntia* subjected to irrigation systems fluctuated between 70.84 - 152.30 µM eq. Trolox /g dry weight and 12.07 - 58.28 µM eq. Trolox /g dry weight, estimated by ABTS and FRAP, respectively (Table 1). The mucilage of the 'Roja Lisa' variety showed the lowest AC values, while the 'Cristalina' variety showed the highest values in both methods. The AC of the cladode extracts of WI plants was higher in all varieties ($p \leq 0.05$), such that the AC of this mucilage was 16 % and 64 % higher, as determined by ABTS and FRAP tests, respectively, compared to mucilage from nopales subjected to CI. Whereas, the antioxidant capacity of WI mucilage compared to mucilage from plants subjected to SI was 9.5 % and 36 % higher (ABTS and FRAP tests, respectively). Therefore, subjecting cactus plants to moderate water stress (SI) could enhance their bioactive properties. Furthermore, this agronomic practice results in a greater production of cladodes and biomass compared to plants experiencing excessive water stress (CI) (Neupane *et al.,* 2021).

Interestingly, the results show that there is a correlation between total polyphenolic content and antioxidant capacity by the ABTS method ($r = 0.96$; $p = 0.0001$) and FRAP method ($r = 0.83$; $p = 0.0001$). This coincided with what was documented by Boutakiout *et al.,* (2018), who found that the antioxidant capacity increased in nopal harvested during drought. Thus, the mucilage of cactus plants grown under drought (WI) or moderate water stress (SI) can serve as an essential source of antioxidants, which could have various applications in the food and pharmaceutical industries. These findings could represent a significant opportunity for cultivating *Opuntia* spp. in semi-arid regions.

Edible coatings

Opacity

The alginate-mucilage film showed an opacity of 1.12 ± 0.10. This result is similar to that reported by Paula *et al.,* (2015) in films based on sodium alginate. The low opacity values of the alginate-mucilage-based film indicate high transparency and a lack of color, making it more likely to be accepted by consumers when applied to food products (Hadi *et al.,* 2022).

Water vapour permeability

The developed film presented a WVP of $2.16 \pm 0.0 \times 10^{-12}$ g m^{-1} h^{-1} Pa^{-1}. This result is lower than those documented by Abdel-Aziz and Salama (2021), in an edible film based on sodium alginate, and in another composed of sodium alginate and *Meyerozyma caribbica* (Iñiguez-Moreno *et al.,* 2021). The alginate-mucilage film developed here could decrease the transfer of water between food and the surroundings, which, in turn, would extend the shelf life of the coated products (Fan *et al.,* 2021).

Mechanical properties

The mechanical properties of edible films indicate the film's ability to withstand food preparation, handling, and storage, helping to preserve food integrity (Marangoni Júnior *et al.,* 2022). The percentage of elongation of the alginate-mucilage film was 12.28 ± 3.04 %, the tensile strength had a value of 0.01 ± 0.00 MPa, and the Young's Modulus was 0.02 ± 0.00 MPa, which are characteristic values of an elastic and flexible edible film. Films prepared with 5% alginate and 0.25 to 1% load of mucilage from 'Algerian' and 'Morado' nopal varieties showed a range of elongation at break of 5-15 %, which is comparable to our evaluated films (Van Rooyen *et al.,* 2023). Yet, the tensile strength of these films was in the range of 8-10 MPa, which is significantly higher than the mechanical stability reported for coatings described here.

Conclusions

The effect of different irrigation systems (WI, SI, and CI) on the functional and bioactive properties of mucilage from several varieties of prickly pear cactus, as well as the technological application of this compound, was investigated. The results of the research are encouraging, as they show that the amount of water contained in the soil affected the functional properties of the mucilage and its bioactive compounds. Overall, plants under limited irrigation produced mucilage with higher WRC, swelling, solubility, TPC, and AC compared to complete irrigation. Likewise, the mucilage of the 'Cristalina' variety stood out for its highest values of TPC, AC, and swelling index. In contrast, the extracts of the 'Roja Lista' variety were distinguished by their high WRC and solubility. The edible films, fortified with nopal mucilage, were prepared and exhibited mechanical properties, opacity, and water vapour permeability characteristic of an edible covering suitable for the preservation of fruits and vegetables susceptible to oxidation and rapid deterioration, such as fresh cut fruits and vegetables.

Therefore, the functional and bioactive properties of nopal mucilage can be enhanced by reducing the nopal plant's water availability. However, subjecting plants to excessive water stress results in lower-quality and fewer cladodes produced in the prickly pear cactus, as well as lower levels of mucilage being produced. Therefore, the use of supplementary irrigation could be a viable alternative cultivation practice for enhancing plant production and, consequently, increasing the production of mucilage with desirable functional and bioactive properties, potentially applicable in the food, pharmaceutical, and chemical industries.

However, while the results are promising, there are several limitations and opportunities for future research. It is important to study factors such as production technology, functionality, environmental benefits, and consumer benefits of cost-effectiveness with regard to the production and application of edible films with added mucilage in the food industry. In addition, evaluate the application of the edible films to a wide range of fruits and vegetables, as different types of products may react differently.

Authors' Contributions

Conceptualization JAZD and JAMV; methodology EALZ, JAAV and VMRG; software EALZ and ESC; validation JAZD and JAMV; formal analysis JAMV and EALZ; investigation JAZD and JAMV; resources JAMV; data curation JAMV and EALZ; writing-original draft preparation EALZ; writing-review and editing JEM and JAMV; visualization EALZ; supervision JAZD and JAMV; project administration JAZD and JAMV; funding acquisition JAMV.

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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