Enseñar la explotación de la tierra, no la del hombre

UNIVERSIDAD AUTÓNOMA CHAPINGO

Unidad Regional Universitaria de Zonas Áridas

Doctorado en Ciencias en Recursos Naturales Medio Ambiente en Zonas Áridas

PHYSICOCHEMICAL, FUNCTIONAL, AND BIOACTIVE COMPOUNDS CHARACTERIZATION OF MUCILAGE FROM IRRIGATED *Opuntia* spp. AND ITS APPLICATION AS AN EDIBLE COATING

CARACTERIZACIÓN FISICOQUÍMICA, FUNCIONAL Y COMPUESTOS BIOACTIVOS DE MUCÍLAGO DE *Opuntia* spp. IRRIGADA Y SU APLICACIÓN COMO RECUBRIMIENTO COMESTIBLE

THESIS

Submitted as a partial requirement to obtain the degree of:

DOCTOR EN CIENCIAS EN RECURSOS NATURALES Y MEDIO AMBIENTE EN ZONAS ÁRIDAS

By:

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PHYSICOCHEMICAL, FUNCTIONAL, AND BIOACTIVE COMPOUNDS CHARACTERIZATION OF MUCILAGE FROM IRRIGATED *Opuntia* spp. AND ITS APPLICATION AS AN EDIBLE COATING

A thesis presented by EDÉN ARELÍ LUNA ZAPIÉN under the supervision of the Advisory Committee indicated, approved by it, and accepted as a partial fulfillment for the degree of:

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USED ABBREVIATIONS

ABTS	2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)
CRAc	Capacidad de Retención de Aceite
CRA	Capacidad de Retención de Agua
DPPH	2.2-diphenyl-1-picrylhydracil
dm	dry matter
FI	Full Irrigation
FRAP	Ferric Reducing Antioxidant Power
FTIR	Fourier Transform Infrared Spectroscopy
NI	Non-Irrigated
OHC	Oil Holding Capacity
RWC	Relative Water Content
SI	Supplemental Irrigation
SwI	Swelling Index
TPC	Total Polyphenol Content
WRC	Water Retention Capacity
WVP	Water Vapor Permeability
WVTR	Water Vapor Transmission Rate

DEDICATED TO

To my father:

The person who gave me life, educated me and was willing to sacrifice everything for my well-being. Today you are no longer with me physically, but your memories and teachings are engraved in my mind and heart until the end of my life. You were always so proud of me and I know that this time would be no exception. Very soon we will embrace again.

To my mother:

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Author of two scientific articles on food preservation and author of a review on nopal mucilage.

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RESUMEN GENERAL

Caracterización fisicoquímica, funcional y compuestos bioactivos de mucílago *Opuntia* spp irrigada y su aplicación como recubrimiento comestible

Las plantas del género *Opuntia* spp. ocupan un lugar preponderante por su capacidad de adaptación a condiciones climáticas extremas. Los cladodios son una fuente importante de mucílago, el cual es un compuesto promisorio para ser utilizado como aditivo agroindustrial. Tres regímenes de riego fueron probados: sin riego (agua de lluvia), riego suplementario donde las plantas fueron irrigadas a capacidad de campo (0.28 m^3 m^{-3}) cuando el contenido del agua en el suelo alcanzó el punto de marchitez permanente (0.14 m^3 m^{-3}) y riego completo (100 % de la evapotranspiración del cultivo) en las variedades de nopal tunero 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' y 'Roja Lisa'. Los cladodios fueron recolectados para extraer el mucílago. El mucílago fue caracterizado en rendimiento, color, composición química, espectroscopia de infrarrojo, viscosidad, masa molar, propiedades funcionales, polifenoles totales y capacidad antioxidante. La estructura del mucílago obtenido correspondió, probablemente a un esqueleto de xiloglucano con ramificaciones de arabinosa. En general el mucílago de plantas 'Amarilla Olorosa' y 'Roja Lisa' cultivadas sin riego produjeron el mayor contenido de mucílago, luminosidad, carbohidratos, fibra, contenido relativo de agua y solubilidad; mientras que paralelamente, el contenido de cenizas fue menor en comparación con el mucílago de las variedades 'Cristalina' y 'Dalia Roja'. El mucílago de las plantas de 'Amarilla Olorosa' sin riego fue el más viscoso. Por otra parte, el polvo de mucílago variedad 'Cristalina' sin riego observó el mayor índice de hinchamiento con altas concentraciones de polifenoles totales y capacidad antioxidante. Por lo tanto, este mucílago, junto con el alginato de sodio, fue usado para la elaboración de una cubierta comestible. Este recubrimiento fue útil para conservar la calidad comercial de mitades de aguacate. Por lo tanto, la restricción de agua en plantas de nopal tunero es una estrategia factible para producir mucílago con características fisicoquímicas relevantes para propósitos agroindustriales.

Palabras clave: *Opuntia* spp.; cladodio; heteropolisacárido; azúcares; antioxidantes; películas de recubrimiento.

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GENERAL ABSTRACT

Physicochemical, functional, and bioactive compounds characterization of mucilage from irrigated *Opuntia* spp and its application as an edible coating

Plants of the genus *Opuntia* spp. occupy a preponderant place due to their ability to adapt to extreme climatic conditions. Cladodes are an important source of mucilage, which is a promising compound to be used as an agro-industrial additive. Three irrigation regimes were tested: non-irrigated (rainwater), supplemental irrigation where plants were irrigated at field capacity (0.28 m^3 m^{-3}) when soil water content reached the permanent wilting point (0.14 m^3 m^{-3}), and full irrigation (100 % of the evapotranspiration of the crop) on 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' and 'Roja Lisa' varieties of prickly pear cactus. The cladodes of these varieties were collected to extract and purify the mucilage. The mucilage was characterized in terms of yield, color, chemical composition, infrared spectroscopy, viscosity, molar mass, functional properties, total polyphenols, and antioxidant capacity. The structure of the mucilage obtained corresponded to a xyloglucan skeleton with arabinose ramifications. In general, the mucilage of 'Amarilla Olorosa' and 'Roja Lisa' plants under no irrigation produced the highest mucilage content, better lightness, carbohydrate, fiber content, relative water content, and solubility; while parallelly, the ash content was lower compared to the mucilage from 'Cristalina' and 'Dalia Roja' varieties. The mucilage of the 'Amarilla Olorosa' plants under no irrigation was the most viscous. On the other hand, the mucilage powder of the non-irrigated 'Cristalina' variety had the highest swelling index with high concentrations of total polyphenols and antioxidant capacity. Therefore, this mucilage, along with sodium alginate, was used to elaborate an edible coating. This coating was useful to preserve the commercial quality of avocado halves. Therefore, water restriction in prickly pear cactus plants of the varieties studied here, is a feasible strategy to produce mucilage with physicochemical characteristics relevant for agro-industrial purposes.

Keywords: *Opuntia* spp.; cladode; heteropolysaccharide; sugars; antioxidants; coating films.

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1. GENERAL INTRODUCTION

The *Opuntia* genus comprises more than 300 wild and domesticated species, 78 of which are endemic to Mexico (Kalegowda et al., 2017; López-Palacios et al., 2012). These plants use a CO_2 fixation system known as crassulacean acid metabolism (CAM plants), which is characterized by nocturnal carbon fixation and high-water use efficiency (Clifford et al., 2002). Cactus pear is a socially and economically important crop in marginal agricultural lands of arid and semi-arid regions worldwide. In 2021, there was 45,320 ha cultivated with cactus pear (*Opuntia* spp.) for fruit consumption in Mexico (SIAP, 2022). However, this plant is also cultivated for animal feed, soil restoration, soil erosion minimization, and to produce mucilage used in both the pharmaceutical and food industries (Gallegos-Vázquez et al., 2011; Varela-Gámez et al., 2014; Vargas-Solano et al., 2022).

Cactus pear is commonly cultivated under rainfed conditions, where rainfall is scarce and erratic, which represents the main limiting factor of productivity in producing areas (Mohamed et al., 2021). These conditions cause the cladodes and fruits to dehydrate, and therefore, this is reflected negatively in fruit yields and fruit quality (Mohamed et al., 2021). It is known that the addition of limited amounts of water to rainfed crops can be a successful production strategy for increasing and stabilizing crop productivity such as cactus pear. This agronomic practice is known as supplemental irrigation (SI) (Zegbe & Palestina, 2020). Neupane et al. (2021) demonstrated increased cactus biomass production under SI conditions. Also, this irrigation system enhanced fruit yield and fruit size (Arba et al., 2018; Van Der Merwe et al., 1997), preserved flesh firmness, and extended the shelf life of cactus pear fruits (Zegbe, 2015; Zegbe & Servín-Palestina, 2020).

Water is the main component in cladodes of *O. ficus-indica* cladodes (80 to 95%) followed by carbohydrates present as monosaccharides (3 to 7%), fiber (1 to 2%), and protein (0.5 to 1%) (Garfias-Silva et al., 2022). Mucilage is the most important heteropolysaccharide constituent (Procacci et al., 2021). This compound is composed mainly of arabinose, galactose, galacturonic acid, rhamnose, and xylose units (Cruz-Rubio et al., 2021). The mucilage is found in the extracellular spaces; however, it is synthesized from the polymerization of several monosaccharides associated with uronic acids. This compound is excreted in the apoplast forming a pocket between the cellular membrane and cell wall. This helps regulate cellular water content at the start of the dehydration process (Haughn & Western, 2012; Nobel et al., 1992). The most important functions of mucilage in plants are maintaining ionic balance in plant cells, transporting and retaining water, frost tolerance, and carbohydrate storage. (Bhurat et al., 2011).

In addition, mucilage has beneficial properties for human health, such as anti-ulcerous (Galati et al., 2001), anti-inflammatory and cytoprotective (Galati et al., 2007), hypoglycemic and hypolipidemic (Herrera et al., 2021), and antioxidant (Jaramillo-Flores et al., 2003). Cladode mucilage has been used to remove water turbidity (Kevin et al., 2006) and heavy metals in both freshwater and wastewater (Nharingo et al., 2015; Vargas-Solano et al., 2022). It has been used also as a coagulant-flocculant agent to treat textile effluents (De Souza et al., 2016), and as an additive for building materials (Aparicio et al., 2019). This compound is used as a food thickener and emulsifier (León-Martínez et al., 2010), as an encapsulant for bioactive compounds (Soto-Castro et al., 2019) and for coating fruits and vegetables (Zambrano-Zaragoza et al., 2014).

In addition to the above, it has been documented that the functional and biological properties of polysaccharides such as mucilage are related to their structure. (Carmona et al., 2021; De Araújo et al., 2021; Rodríguez-García et al., 2007). However, cladode mucilage can be modified in yield and physicochemical properties due to plant species, cladode age, and cladode harvest time (Contreras-Padilla et al., 2016; Du Toit et al., 2019; Rodríguez-González et al., 2014). Nonetheless, the effect of water supply through drip irrigation during the dry season on yield, physical and chemical properties and mucilage composition are unknown.

1.1 Objectives

1.1.1 General objective

To evaluate the influence of the irrigation regime and cactus pear varieties on the physicochemical, functional properties, and bioactive compounds of mucilage and its application as edible coating.

1.1.2 Specific objectives

To characterize the cactus pear mucilage by evaluating its physicochemical, functional properties, and bioactive compounds.

To characterize, prepare, and apply a mucilage-based edible coating to minimally processed avocados.

1.2 Hypothesis

The interaction between irrigation regimes and cactus pear varieties quantitatively and qualitatively enhances the mucilage's physicochemical, functional properties, and bioactive compounds. In addition, the edible mucilage coating will preserve the quality and sensory properties of minimally processed avocados.

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2. LITERATURE REVIEW

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Me es grato informarle que su contribución titulada:

MUCÍLAGO DE NOPAL (Opuntia spp.) Y SU APLICACIÓN COMO ADITIVO ALIMENTARIO: UNA VISIÓN GENERAL

Ha sido aceptada para publicación en un próximo número de la Revista Fitotecnia Mexicana, que en breve sera publicado.

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Agradezco de antemano la atención que se sirva prestar a la presente y aprovecho la ocasión para enviarle un cordial saludo.

ATENTAMENTE

Dra. Micaela de la O Olán
Tesorera de la SOMEFI

Ccp. Dr. Amalio Santacruz Varela, Director de la RFM.

A literature review entitled "Cactus pear mucilage (*Opuntia* spp.) and its application as a food additive: an overview" has been accepted for publication in the Revista Fitotecnia Mexicana.

Cactus pear (*Opuntia* spp.), a plant of the family of *Cactaceae*, is characterized by a green color, the presence of spines, and a branches-structured plant. Around the world, there are more than 300 species of this genus (Galicia-Villanueva et al., 2017). These plants present a CO_2 fixation system called crassulacean acid metabolism (CAM), by which the stomatic opening, and therefore carbon fixation, is nocturnal when both the air temperature and relative humidity are lower than daytime (Nobel & Bobich, 2002). The latter, besides anatomical and morphological changes, has contributed to the adaptation of these plants to drought conditions in arid and semi-arid regions worldwide (Franco-Salazar & Véliz, 2008). Several species of the genus *Opuntia*, since pre-Hispanic times, have been consumed by humans as vegetables and fruit, fodder, and for other uses such as therapeutic, nutraceutical, functional, and industrial (Gallegos & García, 1870). At the present, one of the aspects that have gained the most attention is the hydrocolloids, known as mucilage, which have remarkable industrial and functional applications (Saenz & Cuevas, 2013), in addition to various beneficial human-health effects attributed to this compound (Galati et al., 2007; Souza et al., 2014).

2.1 Composition of mucilage

Mucilage is a dense, thick, viscous, and sticky fluid, which is secreted and stored in the stems of succulent plants. Mucilage is synthesized for water-storage purposes through the secretion of polysaccharides in the extracellular spaces (Archana et al., 2013). The stored water is used during soil water deficit episodes (Nobel & Bobich, 2002). Mucilage plays an important role in keeping the ionic balance of plant cells, frost tolerance, water transport, wound response, among others plant-host-pathogen interaction, and carbohydrate reserves (Bhurat et al., 2011). Besides storing water, mucilage contributes to seed germination of some plants, acts as a membrane thickener, and as food storing (Soukoulis et al., 2018). The accumulation of low-molecular-mass substances in the mucilage, such as

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sugars and proteins, acts as a cryoprotective since these restrict the mobility of intracellular water. However, below -6 °C, when extracellular ice crystals extract water from mucilage cells, irreversible cell breakdown occurs (Goldstein & Nobel, 1994; Nobel & De la Barrera, 2003). In contrast, these plants are high-temperature tolerant, so mature cladode might survive at 65 °C for 60 min (Nobel & De la Barrera, 2003). Yet, the thermal and optimal range for this species is from 6 to 36 °C and from 18 to 20 °C, respectively (Granados-Sánchez & Castañeda-Pérez, 1991).

Mucilage is found in the mucilaginous cells of roots, cladodes, flowers, and fruit. The largest number of mucilaginous cells is found in cladodes, specifically in the parenchyma (where water is stored) and lower in the chlorenchyma (where photosynthesis takes place) (Terrazas & Mauseth, 2002). Mucilage is synthesized in the Golgi apparatus (Trachtenberg & Mayer, 1980). It is produced by dictyosomes, which are packaged in vesicles and transported outside the protoplast, and deposited outside the cell when the vesicles fuse with the plasma membrane (granulocrine secretion). The mucilage cell has a sole thinning and compact primary wall that prevents leaking the mucilaginous material into intercellular spaces. As mucilage builds up into the cell, the protoplast shrinks and collapses, and eventually, the entire cell volume is filled with mucilage (Mauseth, 2008).

The cactus pear mucilage is comprised of long-chain polyelectrolyte molecules is a long-chain polyelectrolyte that possesses functional groups negatively charged throughout the molecule that repel themselves, increasing the viscosity (Trachtenberg & Mayer, 1980). Usually, these molecules exhibit molecular weights ranging from 2.3×10^4 to 4.3×10^6 Da (Medina-Torres et al., 2000). Mucilaginous polysaccharides are mainly comprised of arabinose (10.1-44.0%), xylose (5.1-22.1%), galactose (20.4-33.0%), galacturonic acid (0.18-18.5%), and rhamnose (4.5-15.70%) (Stintzing & Carle, 2005). The mucilage presents a main linear chain with replicates of β-(1-4)-D-galacturonic acid and α-(1-2)-L-rhamnose

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linked with lateral chains of oligosaccharides β-(1-6)-D-galactose bound to O-(4) of L-rhamnose and residues (Espino-Díaz et al., 2010; Sáenz et al., 2004).

Numerous studies have been conducted to evaluate the differences in yield and composition of mucilage monosaccharides in the *Opuntia* species (see Table 1). Particularly, McGarvie and Parolis (1979) exposed that climatic conditions can modify the mucilage content of the plant, which is reflected in low yields during the mucilage extraction (0.06% based on fresh matter).

Furthermore, the monosaccharide composition from *Opuntia* species is attributed not only to the cladode age but also to the pH and composition of the soil (De Oliveira-Ribeiro et al., 2010). Besides, the mucilage yield is also modified by the hydric status and acidity of the cladode (Domínguez-Canales et al., 2011). It has been also seen that as cladodes mature, mucilage yield and soluble fiber decrease (Contreras-Padilla et al., 2016).

Roberts (1945) observed that mucilage from cladodes varies depending on the amount of water the plant receives. Specifically, extremely dry soil produces higher viscous mucilages (Domínguez-Canales et al., 2011). Likewise, Goldstein and Nobel (1991) mentioned that the cladode mucilage content increased in response to soil water content.

This can be attributed to a protective reaction of the plant against abiotic factors. In contrast, De Wit et al. (2017) found that the cladode moisture content (82-90%) has little correlation with mucilage yield. Similarly, Du-Toit et al. (2020) concluded that monthly precipitation was not strongly correlated with the yield, viscosity, pH, conductivity, and malic acid content in extracted mucilage from *O. ficus-indica* and *O. robusta.*

2.2 Mucilage extraction

Currently, cactus pear mucilage can be extracted by different methods to obtain high yields, as well as proposing more efficient methods in terms of time, energy, and environmentally friendly.

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Table 1. Extraction and composition of the mucilage from different *Opuntia* species.

Species	Extraction method	Yield (%) dry matter	Composition	Reference
O. ficus-indica	The pulp was centrifuged at 10,000 rpm, decanted, and acetone precipitated in a mucilaginous pulp: acetone ratio of 1: 2. Then the precipitate was collected, washed with isopropyl alcohol in a volume ratio of 1:1, and finally dried.	-	Arabinose (44.04%) Galactose (20.43%) Xylose (22.13%) Rhamnose (7.92%) Galacturonic acid (6.38%)	(Medina-Torres et al., 2000)
O. ficus-indica	The epidermis was removed, then mixed in a mill. A microwave-assisted extraction was performed at 700 W for 5.15 min, using a liquid: solid ratio of 4.83 mL/g and pH of 11. The extracted mucilage was cooled in an ice bath (4 °C), filtered, and centrifuged at 4000 *g* for 15 min at 4 °C. The filtrate was precipitated using three volumes of 95% ethanol (v/v) at 4 °C overnight. The precipitate was washed three times with ethanol (75% v/v) and then lyophilized.	25.60 dw	-	(Felkai-Haddache et al., 2016)
O. dillenii	The cladodes were cut into small pieces, mixed and stirred with 500 mL of water (45 °C), and stirred for 6 h. The mucilage separated using a muslin-cloth. Viscous filtration was concentrated at a reduced pressure between 40 and 50 °C with a rotary evaporator. The concentrate was precipitated with four volumes of 95% ethanol. The precipitate was washed with anhydrous ethanol, dialyzed for 24 h using a molecular weight cut-off between 12 and 14 kDa, and lyophilized.	6.20 dw	Arabinose (38.80%) Galactose (33.00%) Xylose (5.10%) Rhamnose (15.70%) Glucose (5.10%) Uronic acid (2.50%)	(Kalegowda et al., 2017)
O. spp.	The cladodes were washed, diced, and crushed into a blender with water in a 1:3 ratio (w/v). The suspension was centrifuged and then filtered through a muslin cloth. The mucilage was precipitated using three volumes of 95% ethanol. The mucilage was collected as sediment by centrifugation. The precipitate was washed three times with 95% ethanol and three times with acetone and lyophilized. Subsequently, the mucilage powder was purified by dialysis. Finally, the mucilage was lyophilized and pulverized.	3.86 dw	Arabinose (10.1%) Galactose (25.6%) Xylose (8.4%) Rhamnose (9.8%) Galacturonic acid (18.5%) Glucose (5.2%) Mannose (12. 1%)	(Manhivi et al., 2018)

O. monacantha	The cladodes were de-spined, sanitized, chopped (~1 cm^3), weighed, and crushed. The pulp was mixed with water in a proportion of 1:2 pulp: water. The mixture was stirred and heated mechanically to 80 °C for 30 min. The suspension was filtered and centrifuged (10,000 *g* for 20 min at 20 °C). The supernatant was precipitated with 95% ethanol at a ratio of 1:3 supernatant: ethanol and remained overnight at 4 °C. After precipitation, the white fibrous material (mucilage) was recovered by vacuum filtration. The filtrate was dried in a circulating air oven at 45 °C for 16 h, the resultant was grounded and sieved with a 60 mesh (standard granulometry ≤ 250 µm).	14 dw	Arabinose (11.60%) Galactose (20.84%) Xylose (6.64%) Rhamnose (4.50%) Galacturonic acid (0.18%) Glucose (4.85%) Glucuronic acid (15.22%)	(Dick et al., 2019)

The mucilage is commonly extracted by maceration followed by filtration and precipitation steps, being water the solvent most used. Additionally, several solvents such as ethanol, isopropyl alcohol, and acetone have been used also for the precipitation-step (Bayar et al., 2016; Cai et al., 2008; McGarvie & Parolis, 1979; Medina-Torres et al., 2000; Sepúlveda et al., 2007).

In the last decade, microwave-assisted extraction has been considered one of the procedures most used in mucilage extraction (Felkai-Haddache et al., 2016). This method has shown several advantages such as a significant reduction in processing time and the use of environmentally friendly solvents, reducing processing costs. In particular, Thirugnanasambandham et al. (2015) reported that an increase in microwave power significantly improves sample solubility in favor of greater extraction efficiency. Increased irradiation energy improves solvent penetration into the plant tissues and efficiently releases materials due to molecular interaction with the electromagnetic field. The microwaves provide a rapid energy transfer to the solvent and plant tissue to achieve the dissolution of components to be extracted (Yan et al., 2010). It is also known that the energy increasing, simultaneously stimulates the rotation of the dipoles generating heat in the reaction mixture, and finally, this is reflected in the polysaccharide yield

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(Maran et al., 2013). Therefore, it has been observed that the mucilage yield increases linearly and positively with the extraction time (Felkai-Haddache et al., 2016). Thus, an increase in the extraction time increases the reactive site to the effective extraction process, and therefore, this is translated into higher mucilage yields (Maran et al., 2013). In contrast, long extraction times combined with high power led to the degradation of the polysaccharides (Thirugnanasambandham et al., 2015).

Another important factor for optimal mucilage extraction is the solid: liquid ratio. Particularly, the use of a small solid: liquid ratio leads to obtaining poor extraction yield of polysaccharides and could result in high costs in the extraction process. Therefore, it is imperative to know the appropriate ratio of raw material and water to extract the highest content of polysaccharides (Samavati, 2013; Yin & Dang, 2008). Thus, an increase in the proportion of water can increase the diffusivity of the solvent in cells and thus improve the desorption of polysaccharides from cells (Volpi, 2004). In other studies where different proportions of water-raw material were tested, the extraction yields became asymptotic (Bendahou et al., 2007).

In mucilage extraction, high yields are commonly associated with pH neutralization for extraction. This could be attributed to the induced dissociation of the acidic group (-COOH) of polysaccharides and the repulsion between negative loads (OH-). This can also increase the solubility of polysaccharides in water and, consequently, improves the mucilage yield (Liu & Fang, 2002). On the other hand, the mucilage yield tends to decrease when alkaline pH is used since this affects the polysaccharides' solubility (Yang et al., 2015).

2.3 Properties of cactus pear mucilage

2.3.1 Physical properties

The physical-chemical properties of *Opuntia* mucilage are highly variable because they are affected by a series of factors such as soil chemical characteristics, geographical location, environmental conditions, cladode age, and the genetic load of these plants (Nharingo & Moyo, 2016; Sepúlveda et al., 2007).

Solubility

Solubility is an important factor since the maximum functionality of any hydrocolloid is achieved after its complete dissolution in water (Laaman, 2011). It has been reported that mucilage produces viscous solutions in cold water, it is also soluble in hot water, and lesser soluble in other solvents such as citric acid, hydrochloric acid, and sodium hydroxide (Kalegowda et al., 2017). Temperature plays a key role in the solubility of mucilage since to some extent, the association between temperature and mucilage solubility is linear. Particularly, Dick et al. (2019) observed greater solubility of mucilage at temperatures higher than 60°C. Thus, the mucilage solubility in water is related to the strength of the interactions between the mucilage polysaccharides and the water hydrogen bonds driven by hydrophilic groups along the polymer chain (Doublier & Cuvelier, 2006). Besides, the heating process reduces particle aggregation, which in turn favors the diffusion of water to the sample mass (Alpizar-Reyes et al., 2017).

Density and electrical conductivity

It has been established also that the density of the mucilage *O. cochenillifera* and *O. monacantha* ranges from 0.785 to 1.05 g·mL^{-1} (Dick et al., 2019). On the other hand, a positive correlation between mucilage concentration and electrical conductivity has been indicated (Monrroy et al., 2017). Electrical conductivity variations at different concentrations of mucilage can be attributed to the presence of a greater number of divalent and monovalent ions, which increase conductivity (Gebresamuel & Gebre-Mariam, 2012).

Microstructure

In the last years, numerous studies have been conducted to observe the microstructure of cactus pear mucilage. Interestingly, Dick et al. (2019) observed that mucilage exhibited irregular-shaped particles with a wide range of sizes. On

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the other hand, the mucilage powders have shown a high apparent porosity, giving it a spongy appearance similar to that of hygroscopic materials.

In the study conducted by Madera-Santana et al. (2018), electronic scanning micrographs were performed with *O. spinulifera* mucilage powder showing a wide distribution of the particle sizes as well as irregular morphology of them. Also, larger particles were able to display aggregations with smaller particles, due to the electrostatic charge among particles. Kalegowda et al. (2017) observed irregularly shaped aggregates and rough surfaces in the mucilage extracted from *O. dillenii*. However, Otálora et al. (2015) observed spherical and non-agglomerated particles with a uniform appearance in mucilage cladodes of *O. ficus-indica*. The larger particles were able to display aggregations with those smaller, this due to the electrostatic charge among particles. These authors attributed this phenomenon to the drying process of electronic sweeping microphotographs of mucilage extracted from the parenchyma and collenchyma of *O. robusta*. These have shown a greater level of aggregation of small particles in the mucilage extracted from the parenchyma than that extracted from the chlorenchyma. The mucilage particles extracted from the collenchyma are mainly observed as aggregates of irregular shapes and dimensions and they are fibrous-natured (Bernardino-Nicanor et al., 2018) (Figure 1). Thus, the shape and microstructure of mucilage powder depend on the method of extraction, purification, drying, and pulverizing of the sample (Dick et al., 2019).

Color

Another important characteristic of mucilage is its color. The mucilage powder has a high luminosity (L*) that matches a light color, with the chromatic coordinates in the yellow-green spectrum (b* positive component and component a* negative) (Dick et al., 2019; Sepúlveda et al., 2007). In the cladode samples, during the precipitation process with ethanol, chlorophyll residues are dissolved, generating depigmented mucilage (Dick et al., 2019).

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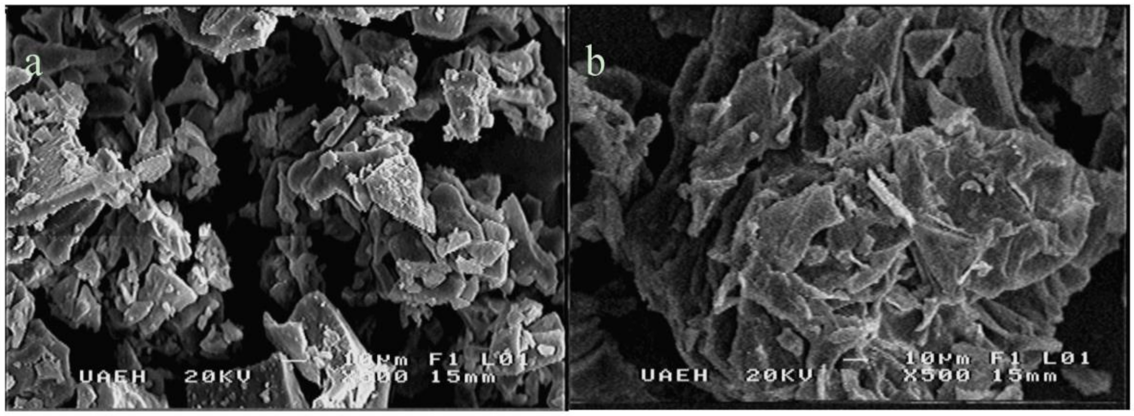

Figure 1. SEM micrograph of *Opuntia robusta* mucilage: (a) extracted from the chlorenchymatous tissue; (b) extracted from the parenchymatous tissue.

(Bernardino-Nicanor et al., 2018)

2.3.2 Chemical properties

The protein content in mucilage is an important chemical parameter to be characterized. The protein values fluctuate depending on the genetic diversity of the *Opuntia* genus. Gebresamuel and Gebre-Mariam (2012) in a proximal mucilage study (*Opuntia* spp.), observed 6.82% protein; while in *O. spinulifera* the protein content of mucilage powder was 9.8% (Madera-Santana et al., 2018). In *O. monacantha* a 3.6% of the protein was observed (Dick et al., 2019), while in *O. ficus-indica* the protein values were found between 6.1 and 7.9% (Sepúlveda et al., 2007).

The presence of prolamins, with high content of glutamine and proline, has been identified (Du Toit et al., 2018). In addition, the presence of small protein fractions (10 kDa) indicates that mucilage of *O. ficus-indica* contains albumin. These protein fractions give to the mucilage the ability to form emulsions. This is possible because they reduce the interfacial tension by increasing the emulsion stability (Du Toit et al., 2018).

In relation to the lipid content in the cactus pear mucilage, Dick et al. (2019) recorded lipid values around 1.2% regarding to dry matter in *O. monacantha*

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mucilage; while lipid content was as low as 0.09% in *O. ficus-indica* (Rodríguez-González et al., 2014) or 0.61% (Rivera-Corona et al., 2014) and as high as 2.3% (Espino-Díaz et al., 2010). The predominant fatty acids in *O. robusta* and *O. ficus-indica* mucilage are linoleic, oleic, palmitic, and stearic, these four acids constitute approximately 98.2% of the total fatty acids in the mucilage (Du Toit et al., 2018). Ash is the product of the combustion of the material comprised by non-combustible inorganic substances, such as mineral salts. Thus, mucilage is an excellent source of essential minerals such as calcium, phosphorus, magnesium, iron, potassium, zinc, and copper, being calcium the most abundant mineral in mucilage (Monrroy et al., 2017). The ash content is a useful property because indicates the degree of mineral interaction in the structure that contributes to the functional properties of polysaccharides (Amid et al., 2012).

In a study carried out by Espino-Díaz et al. (2010), they pointed out mucilage ash values approximately of 20.1%; while Rodríguez-González et al. (2014) reported 16% of ashes in *O. ficus-indica* cladodes, but Sepúlveda et al. (2007) mentioned a range of 34.9% to 39.1%. By contrast, Madera-Santana et al. (2018) reported a value as low as 1.21% of ashes (Aquino et al., 2009). Granados-Sánchez and Castañeda-Pérez (1991) mentioned that cladode ash values can be attributed to high soil salinity and soil fertility. It has also been observed that high ash content in the mucilage may be attributed to the highest ash content of the initial raw materials and the absence of dialysis purification during the mucilage extraction process (Bayar et al., 2016). On the other hand, Wang et al. (2014) observed that the ash content decreased as the temperature of mucilage extraction increased.

2.3.3 Physicochemical properties

The pH is an important factor that determines the ability of mucilage to be used like supplements or food in some products. In the study performed by Kalegowda et al. (2017), the extracted solution from mucilage (1% w/v) turned out to be neutral (7.10). On the contrary, the pH of mucilage solution extracted from O. *cochenillifera* recorded slightly acidic pH values between 4.8 and 5.0, indicating the presence of COOH- radicals contained in the mucilage structure, typically uronic acids (Monrroy et al., 2017).

2.3.4 Functional

The Water Retention Capacity (WRC) is a functional property that indicates the ability of mucilage to retain water. Interestingly, it has been observed a wide range of WRC depending on plant variety; i.e.: values of 7.8 $g \cdot g^{-1}$ have been observed in *O. ficus-indica* (Bayar et al., 2016) mucilage while in *O. cochenillifera* has been around 2.8 $g \cdot g^{-1}$ (Monrroy et al., 2017). High WRC values have been associated with, both, high concentrations of free hydroxyl groups, and high calcium concentrations in the mucilage structure (Prajapati et al., 2013).

On the other hand, it has been observed that the growth periods of the cladodes affect the WRC of the *O. ficus-indica* and *O. robusta* mucilage. For instance, samples collected in April registered values of 3.6 $mL \cdot g^{-1}$, while those collected in August presented values around 15.7 $mL \cdot g^{-1}$ of WRC. So, the low WRC values observed in April may be because the powdered mucilage had a low content of soluble solids, low pH, high conductivity, and low viscosity (Du Toit et al., 2019).

The swelling capacity determines diverse applications of polysaccharides. For example, the mucilage extracted from *O. dillenii* has exhibited a swelling capacity of 20%. This could be related to several factors such as the presence of hydroxyl groups, high galactose units in the extracted polysaccharides and the extraction method used (Kalegowda et al., 2017). Du Toit et al. (2019) has reported values between 1.53 and 2.33 $g \cdot g^{-1}$.

The oil holding capacity of mucilages varies regarding to the species. In particular, mucilage from *O. cochenillifera* has exhibited values of 1.8 g $oil \cdot g^{-1}$ sample (Monrroy et al., 2017) whereas *O. ficus-indica* mucilage showed an OHC of 1.3 $g \cdot g^{-1}$ (Bayar et al., 2016). This mucilage property has been associated with both the hydrophilic character and general load density of this hydrocolloid (Elleuch et al., 2011).

Due to hydrophilic capacity, mucilage can act as a stabilizer for oil in water emulsions, forming a layer around each oil drop, and therefore, delaying the fusion of globules (Prajapati et al., 2013). The mucilage stability could be related to both a low-temperature mucilage extraction (<45 °C) and the extract fractions contaminated by other components such as ferulic acid, proteins, and minerals (Lv et al., 2013). In addition, the methyl and acetyl groups, in the main chain, have a positive effect on emulsion stability (Chen et al., 2016). On the other hand, acidic sugars such as uronic acids have been reported to possess surfactant properties (Razavi et al., 2016).

While foaming ability is the amount of effervescence formed immediately after stirring, foam stability is determined at time intervals. Foaming ability is mucilage concentration-dependent, for instance, 100% of foaming capacity was reached with a 1% solution of extracting mucilage from *O. monacantha* (Dick et al., 2019). In contrast, the mucilage foam stability, in terms of volume, progressively decreased over time. So, mucilage foam at 0.5% and 1% concentrations started with 51.5% and 72.7% at 5 min and ended with 25.5% and 46% after 2 h, respectively (Dick et al., 2019). On the other hand, mucilage extracted from *O. robusta* showed a foam volume of 100% at 10 s after shaking, but the foam bubbles were large, unstable, and disappeared instantly. Also, mucilage samples with a greater total soluble solids content had higher foam formation capacity (Du Toit et al., 2019).

2.3.5 Bioactive compounds and antioxidant capacity

Bioactive substances such as phenolics, stilbenes, flavonoids, tannins, and lignins are important mainly due to the beneficial effects on human health associated to its intake, such as antioxidant activity (Galanakis, 2016; Pandey & Rizvi, 2009).

Manhivi et al. (2018) found that most phenolic compounds in mucilages were esterified (phenolic compounds tied). The presence of esterified phenolic

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compounds may allow the enzymatic modification of mucilages by the lactase enzyme, which is an enzyme of the phenol-oxidase type that improves the functional properties of mucilage.

It has also been observed that the antioxidant capacity of *O. ficus-indica* mucilage at 20 mg·mL^{-1}, showed inhibition of 100% using 2.2-diphenyl-1-picrylhydracil (DPPH) (Bayar et al., 2016). However, mucilage antioxidant capacity decreases when the mucilage extraction is carried out at a high temperature (55-65 °C) (Jouki et al., 2014). According to Zeng et al. (2016), the antioxidant activity of cladode extracts could be associated with hydroxyl and carboxylate groups, which transfer electrons to scavenge free radicals.

Pancreatic lipase is one of the most studied mechanisms for finding the potential efficacy of natural products as an anti-obesity drug (Birari & Bhutani, 2007). This enzyme has been studied as an anti-lipase. The mucilage of *O. dillenii* at 1.25 mg mL^{-1} concentration inhibited this enzyme (Kalegowda et al., 2017). This inhibitory activity is because the mucilage binds to the enzyme causing conformational changes which decrease the enzyme-substrate binding capacity (Hassan, 2009). Uebelhack et al. (2014) demonstrated that cactus fiber is linked to dietary fat that reduces fatty acid absorption, eventually leading to reduced body weight in humans.

2.3.6 Rheological

It has been observed that the viscosity of *O. dillenii* mucilage decrease as the shear rate increase, confirming a non-Newtonian shear-thinning flow behavior (Kalegowda et al., 2017). This pattern has also been observed in *O. ficus-indica* mucilage (Contreras-Padilla et al., 2016; Medina-Torres et al., 2000). This non-Newtonian shear-thinning behavior has been attributed to the occurrence of high molecular-weight compounds in mucilages (Sepúlveda et al., 2007). This is because the molecules of the polymer chain align and therefore, there is lesser interaction among adjacent polymer chains (Zhang et al., 2013).

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The intrinsic viscosity is another important rheological parameter to be characterized. This variable measures the polymer's capability to improve the fluid viscosity of a substance. At the same time, the viscosity is related to the physicochemical properties of the polymer such as its molecular weight, polymer chain shaping, and the type of solvent used during the mucilage extraction (Jindal et al., 2013a, 2013b). According to Mark Houwink's relationship, intrinsic viscosity increases as so does the molecular weight of the polymer chain (Razavi et al., 2016).

Mucilage viscosity is also modified by temperature extraction. The mucilage viscosity increases at low temperatures and decreases at high temperatures (Medina-Torres et al., 2000).

Mucilage viscosity is also affected by the pH of the aqueous solution, which increases as pH increases (Du Toit, 2019; Medina-Torres et al., 2000). In both *O. ficus-indica* and *O. robusta* mucilages, the most acidic one had the lowest viscosity. Furthermore, an association has been observed between low viscosity and high content of malic acid (Du-Toit et al., 2020).

Additionally, the constant viscosity shearing depends on the ion strength, as the ion strength increases, the viscosity decreases. This behavior is most noticeable when using divalent ions (Medina-Torres et al., 2000). In this respect, Du Toit (2019) mentioned that monovalent ions have little ion strength, but divalent and trivalent ones had the greatest influence on the mucilage viscosity. At a zero ionic strength, a negative charge produces a strong intermolecular repulsion and therefore a more expanded mucilage molecule. This may explain the greater mucilage viscosity in deionized water. Besides, the addition of positive ions (Ca^{2+}, Na^{+}, K^{+}) reduces the repulsion and expansion of the molecule resulting in a significant viscosity reduction (Medina-Torres et al., 2000).

Mucilage, with great L-arabinose contents, could generate also great viscosity suspensions when it is suspended in water. This is because the functional groups of the L-arabinose molecule can interact in an intermolecular way. Uronic acid is also a great important molecule for generating viscous solutions, because carboxylic groups can interact either with water molecules or other cations, like those of calcium (Rodríguez-González et al., 2014).

2.4 Applications of mucilage

Some of the medicinal properties of cactus pear cladodes have been associated with the mucilaginous material contained in them. Saenz and Cuevas (2013) pointed-out that mucilage could be potentially used for gastric mucosa treatment and other gastric discomforts.

The ability of mucilage to form molecular networks and retain large amounts of water makes it a powerful hydrocolloid of interest to the chemical and cosmetic industries. Mucilage has also been evaluated in the water clarification due to the retention capacity of different particles dispersed in cloudy water. There is evidence that mucilage of *O. ficus-indica* has been used as an auxiliary natural coagulant aid to remove copper species. Therefore, it can be used as a chemical coagulant substitute for water treatment (Adjeroud et al., 2018). In addition, it has been tested as an emulsifying agent (Garti, 1999) or flocculant (Andrew-Young et al., 2006), because it can be used to modify the rheological and dispersion characteristics of emulsions (Quinzio et al., 2018).

Besides, due to its sugar composition, mucilage can attract or retain certain types of substances positively charged; thus, *O. cochenillifera* mucilage has shown the ability to remove textile dyes from aqueous solutions (Monrroy et al., 2017).

The mucilage exhibits good foaming and stability properties. Therefore, the mucilage could be used as an egg substitute in some products such as bakery items and ice creams. It could also be used as a foam stabilizer in carbonated drinks. Therefore, these foaming properties could be addressed to develop egg-free products, benefiting the egg-allergic consumer or to meet the demand for vegan products (Dick et al., 2019).

Other mucilage applications include their use in foods such as flavoring, fat

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substitutes (Sáenz et al., 2004), and edible coatings to extend the shelf life of highly perishable fruits (Del-Valle et al., 2005).

2.4.1 Mucilage as a food additive

Research in *Opuntia* mucilage has found various applications in areas such as the food industry, cosmetics, pharmaceuticals, construction, and the environment, among others. For example, mucilage can be used to modify the rheological and dispersion characteristics of emulsions (Quinzio et al., 2018). This hydrocolloid has been successfully added as a stabilizer in various products as mayonnaise achieving a product with similar characteristics to commercial ones (Bernardino-Nicanor et al., 2015; Du Toit, 2019). It has been used also in confectionery products elaborating marshmallows (Du Toit et al., 2016) (Table 2).

Table 2. Use of mucilage from different species of cactus pear as a food additive.

Use	Species	Product added	Main results	Reference
Emulsifier	*O. robusta*	Mayonnaise	A product similar to mayonnaise was formulated with similar characteristics to commercial mayonnaise prepared with egg yolk adding 26.8% oil, 62.5% mucilage, and 10.7 % whey protein, with a stable shelf life at 25 °C.	(Bernardino-Nicanor et al., 2015)
	O. robusta	Mayonnaise	The mucilage was applied successfully in mayonnaise products to replace up to 50% egg yolk and 30% oil. The sensory acceptance was greater than that of the control. The mucilage powder can contribute to the nutritional and textural quality of food products like mayonnaise.	(Du Toit et al., 2019)
Stabilizer	*O. ficus-indica*	Marshmallows	In the elaboration of marshmallows, the addition of mucilage could replace gelatin. The treatment (75% mucilage, 12.5% xanthan gum, and 12.5% agar) had the best results in consistency, texture, gel softness, shearing, color, and water activity.	(Du Toit et al., 2016)

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	O. ficus-indica	Dehydrated egg	Eggs were spray-dehydrated either with cladode mucilage or maltodextrin solutions. The former solution induced the highest thermal and mechanical stable product and drying performance. It also had the best uniform and definite sphere-shaped morphology. Thus, the mucilage may be suggested as a thickener agent.	(Medina-Torres et al., 2017)
Encapsulation material	*O. monacantha*	Goji berry zeaxanthin	This study revealed that mucilage is an excellent material to be used as encapsulating agent. The mucilage preserved the zeaxanthin content during the storage period.	(De Campo et al., 2018)
	O. ficus-indica	Yellow-orange prickly pear pulp	Microparticles of orange-yellow prickly pear pulp were encapsulated with a mixture of mucilage and maltodextrin. After 28-day storage, they kept their color. This indicated the mucilage's effectiveness as an encapsulating material.	(Carmona et al., 2021)
Nutraceutical	*O. monacantha*	Gluten-free cracker	The study was oriented to elaborate gluten-free biscuits using mucilage. The cookie mucilage-prepared exhibited similar characteristics compared with the control treatment (commercial hydrocolloids-prepared). The experimental product enhanced the total phenolic content and antioxidant activity, and it had the highest consumer acceptability.	(Dick et al., 2020)
	O. ficus-indica	Bread	Mucilage-added to bread showed acceptable sensorial attributes. The addition of mucilage also improved the bioactive compounds in the bread.	(Liguori et al., 2020)
Coating material	*O. ficus-indica*	Guava	Unprocessed guavas were coated with mucilage films. After eight-days of storing at room temperature, the fruit maintained skin color, flesh firmness, and the total concentration of soluble solids.	(Zegbe et al., 2013)
	O. ficus-indica	Tomato	Fresh tomatoes coated with chitosan and mucilage films had a strong antifungal effect against *Rhizopus stolonifer* either *in vitro* or *in situ* conditions. The treated fruit increased its shelf life.	(Olicón-Hernández a et al., 2019)
Others	*O. ficus-indica*	Dehydrated banana slices	Banana slices treated with mucilage, citric acid, and sodium bisulfite, exhibited less enzymatic browning compared with untreated samples during the drying process. The	(Aquino et al., 2009)

		mucilage formed a protective layer on the surface of the dehydrated banana slices and gave it a shiny effect. It also preserved the product quality during storage period.	
O. ficus-indica O. atropes	Raw milk	The raw milk treated with 0.5% of mucilage reduced the bacterial counts of aerobic mesophiles and total coliforms. This was achieved regardless of the species of cactus pear mucilage and its physical mucilage presentation (dehydrated or liquid).	(Ortiz-Rodríguez et al., 2016)

Cactus mucilage has also been used as a coating material for fruit and vegetables. Natural and edible coatings increase the shelf life of products and preserve sensory characteristics during storage. This hydrocolloid has been used to coat young cladodes as vegetables (called nopalitos) (González, 2011), minimally processed apples (Zambrano-Zaragoza et al., 2014), figs (Allegra et al., 2017), blackberries (Nájera-García et al., 2018), and tomatoes (Olicón-Hernández et al., 2019). Also, cactus pear mucilage combined with antioxidants, such as citric acid and sodium bisulfite, may be an alternative to decrease enzymatic darkening (Aquino et al., 2009) and it can also act as an antimicrobial agent (Ortiz-Rodríguez et al., 2016).

Considering that mucilage is an abundant resource in arid and semi-arid areas, easy to extract, economical, and with similar properties to commercial hydrocolloids, it is a feasible natural resource to be used in food applications.

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3. MUCILAGE YIELD, COMPOSITION, AND PHYSICOCHEMICAL PROPERTIES OF CULTIVATED CACTUS PEAR VARIETIES AS INFLUENCED BY IRRIGATION

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Mucilage Yield, Composition, and Physicochemical Properties of Cultivated Cactus Pear Varieties as Influenced by Irrigation

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Abstract: *Opuntia* spp. plants occupy an important socioeconomic role in arid and semiarid zones where water is scarce. Irrigation increases the productivity of these plants; however, its effect on the yield, composition, and physicochemical properties of the mucilage is unknown. Three irrigation regimens were tested: non-irrigated (rainwater), supplemental irrigation (irrigation between field capacity (0.28 m^3 m^{-3}) and permanent wilting point (0.14 m^3 m^{-3})), and full irrigation (100% of crop evapotranspiration), on the four cactus pear varieties ('Amarilla Olorosa' (*Opuntia* spp.), 'Cristalina' (*Opuntia albicarpa* Scheinvar), 'Dalia Roja' (*Opuntia* spp.), and 'Roja Lisa' (*O. ficus-indica* (L.) Mill)). Irrigation regimens were applied during the dry season (March to June in the northern hemisphere). Composite samples of cladodes per replicate and treatment were collected for mucilage extraction. The mucilage was characterized for yield, color, chemical composition, infrared spectroscopy, viscosity, and molar mass. The combination with the greatest yield was 'Amarilla Olorosa' with no irrigation (22.2%), while the least yield was from 'Cristalina' undergoing full irrigation (12.2%). In general, non-irrigated plants yielded more mucilage, their color was brighter and less green, and they had more protein and fiber. The viscosity and molar mass were greatest in non-irrigated plants. Total carbohydrate content was similar between non-irrigated and supplementally irrigated plants. Thus, for the cactus pear varieties studied here, either no irrigation or supplemental irrigation could be a feasible strategy to produce mucilage with good characteristics for agro-industrial and pharmaceutical use.

Keywords: *Opuntia* spp.; cladode; chemical composition; sugars; heteropolysaccharide; viscosity

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1. Introduction

Cactus pear as a crop is commonly grown under rainfed conditions in areas where rainfall is scarce and erratic. Water is the main limiting factor for its productivity when grown for human consumption (tender cladode and fruit) in arid and semi-arid regions [1]. These conditions induce dehydration of both fruit and cladodes [2]. However, when water is applied using supplemental drip irrigation, both fruit and cladode yields improve significantly [3]. Among other cultural practices, these plants are pruned annually, yielding between 10 and 15 t ha^{-1} of fresh cladodes. Since 45,320 ha in Mexico are cultivated with cactus pear [4], this vegetative material could be used potentially for agro-industrial or other purposes [5].

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3.1 Resumen

Las plantas de *Opuntia* spp. ocupan un papel socioeconómico importante en zonas áridas y semiáridas donde el agua escasea. El riego aumenta la productividad de estas plantas; sin embargo, se desconoce su efecto sobre el rendimiento, composición y propiedades fisicoquímicas del mucílago. Se probaron tres regímenes de riego: sin riego (agua de lluvia), riego suplementario [riego entre la capacidad de campo (0.28 m^3 m^{-3}) y el punto de marchitamiento permanente (0,14 m^3 m^{-3})] y riego completo (100 % de la evapotranspiración del cultivo), sobre cuatro variedades de nopal 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' y 'Roja Lisa'. Los regímenes de riego se aplicaron durante la estación seca (marzo a junio en el hemisferio norte). Se recolectaron muestras compuestas de cladodios por réplica y tratamiento para la extracción de mucílago. El mucílago se caracterizó por rendimiento, color, composición química, espectroscopia infrarroja, viscosidad y masa molar. La combinación de mayor rendimiento fue 'Amarilla Olorosa' sin riego (22.2%), mientras que la de menor rendimiento fue 'Cristalina' con riego completo (12.2%). En general, las plantas sin riego produjeron más mucílago, su color fue más brillante y menos verde, y tenían más proteína y fibra. La viscosidad y la masa molar fueron mayores en las plantas sin riego. El contenido total de carbohidratos fue similar entre las plantas sin riego y con riego suplementario. Por lo tanto, para las variedades de nopal estudiadas aquí, la ausencia de riego o el riego suplementario podrían ser una estrategia factible para producir mucílago con buenas características para uso agroindustrial y farmacéutico.

Palabras clave: *Opuntia* spp, cladodio, composición química, azúcares, heteropolisacárido, viscosidad.

3.2 Abstract

Opuntia spp. plants occupy an important socioeconomic role in arid and semiarid zones where water is scarce. Irrigation increases the productivity of these plants; however, its effect on the yield, composition, and physicochemical properties of the mucilage is unknown. Three irrigation regimens were tested: non-irrigated (rainwater), supplemental irrigation [irrigation between field capacity (0.28 m^3 m^{-3}) and permanent wilting point (0.14 m^3 m^{-3})], and full irrigation (100% of crop evapotranspiration), on the four cactus pear varieties 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' and 'Roja Lisa'. Irrigation regimens were applied during the dry season (March to June in the northern hemisphere). Composite samples of cladodes per replicate and treatment were collected for mucilage extraction. The mucilage was characterized for yield, color, chemical composition, infrared spectroscopy, viscosity, and molar mass. The combination with the greatest yield was 'Amarilla Olorosa' with no irrigation (22.2%), while the least yield was 'Cristalina' undergoing full irrigation (12.2%). In general, non-irrigated plants

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yielded more mucilage, their color was brighter and less green, and they had more protein and fiber. The viscosity and molar mass were greatest in non-irrigated plants. Total carbohydrate content was similar between non-irrigated and supplementally irrigated plants. Thus, for the cactus pear varieties studied here, either no irrigation or supplemental irrigation could be a feasible strategy to produce mucilage with good characteristics for agro-industrial and pharmaceutical use.

Keywords: *Opuntia* spp, cladode, chemical composition, sugars, heteropolysaccharide, viscosity.

3.3 Introduction

Cactus pear as a crop is commonly grown under rainfed conditions in areas where rainfall is scarce and erratic. Water is the main limiting factor for its productivity when grown for human consumption (tender cladode and fruit) in arid and semi-arid regions (Mohamed et al., 2021). These conditions induce dehydration of both fruit and cladodes (Zegbe & Palestina, 2020). However, when water is applied using supplemental drip irrigation, both fruit and cladode yields improve significantly (Van Der Merwe et al., 1997). Among other cultural practices, these plants are pruned annually, yielding between 10 and 15 t ha^{-1} of fresh cladodes. Since 45,320 ha in Mexico are cultivated with cactus pear (SIAP, 2022), this vegetative material could be used potentially for agro-industrial or other purposes (Zegbe, 2020).

Cactus pear cladodes contain a heteropolysaccharide compound called mucilage of great agro-industrial importance (Procacci et al., 2021). This macromolecule is composed mainly of arabinose, galactose, galacturonic acid, rhamnose, and xylose units (Trachtenberg & Mayer, 1981). The most important functions of mucilage in cactus pear plants are to maintain the ionic balance in plant cells, water transport and retention, frost tolerance, and carbohydrate storage (Bhurat et al., 2011). Recently, mucilage has gained importance in the agroindustry due to its multiple uses and applications (Soto-Castro et al., 2019). Nevertheless, the yield and physicochemical properties of the mucilage are influenced by the cactus

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pear variety, cladode age, and weather conditions (Contreras-Padilla et al., 2016; Du Toit et al., 2019; Rodríguez-González et al., 2014). Nevertheless, studies of the effect of drip irrigation supply on mucilage properties have not been conducted so far, which can have positive implications for their uses in the food and pharmaceutical industry and other industrial applications worldwide. Therefore, this study tested the hypothesis that irrigation would modify the yield, composition, and physicochemical characteristics of mucilage extracted from cladodes of four cactus pear varieties. This study included the color, viscosity, and molar mass of the mucilage because of their importance for much more diverse industrial uses.

3.4 Material and methods

3.4.1 Experimental site

The experiment was conducted from 2018 to 2020 at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Zacatecas (latitude 22°54'N, longitude 102°39'W, elevation 2,197 m). The climate of the area is semi-warm, with a mean annual temperature of 14.6 °C, and it receives 416 mm of annual precipitation. The average annual evaporation is 1,609 mm. The orchard soil has a loam texture with an organic matter content of 1.73 % and a pH of 7.75.

3.4.2 Plant material and experimental process

This research included four cactus pear varieties 'Amarilla Olorosa' (*Opuntia* spp.), 'Cristalina' (*Opuntia albicarpa* Scheinvar), 'Dalia Roja' (*Opuntia* spp.), and 'Roja Lisa' (*O. ficus-indica* (L.) Mill).

The drip-irrigation treatments were: non-irrigated (NI) as a control, supplemental irrigation (SI), and full irrigation (FI). NI plants received only rainfall. SI plants received irrigation at field capacity (CC = 0.28 $m^3\ m^{-3}$) when the soil water content (θ) was close to the permanent wilting point (PMP = 0.14 $m^3\ m^{-3}$). The θ was determined weekly, before the next irrigation. FI plants received 100% of the crop

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evapotranspiration weekly, estimated through a water balance. During the drought period (February to May), the Adcon Telemetry System weather station, located 1.8 km from the experimental site, recorded the weather conditions.

The experiment was conducted in a randomized complete block design with factorial arrangement in the treatments (three irrigation levels × four cactus pear varieties), with three replications. The experimental unit consisted of nine plants. The sampling of reproductive cladodes (one-year-old) consisted of collecting healthy, mechanically undamaged cladodes and was done on May 29, 2019. Cumulative water applied up to cladode sampling was 91, 154, and 196 mm for NI, SI, and FI plants, respectively. The water supply in the NI plants was due to the rainfall recorded during the same experimental period.

3.4.3 Mucilage extraction

The cladodes were manually de-spined and sanitized with chlorinated water at 200 ppm. The chlorenchyma was removed, while the hydrenchyma was cubed into ~1 cm^3 pieces. Approximately 100 g tissue cubes were soaked in distilled water (1:10) and liquefied for 45 s. The pH of the mixture was adjusted to 7.00 ± 0.1 with 5 N NaOH, heated at 50 °C with constant stirring for 16 h, and filtered through a sieve. The filtrate was mixed with 96% (v/v) ethanol in a 1:2 ratio. The mixture was stirred for 2 h at room temperature and kept at 4 °C for 24 h until separation. The supernatant was decanted and the precipitate was concentrated in a rotary evaporator (BUCHI R-210, Flawil, Switzerland), lyophilized, and vacuum-packed (Figure 2) (Madera-Santana et al., 2018). The mucilage yield was expressed as a percentage by the ratio between the dry weight of the extracted mucilage and the dry weight of the samples (w/w).

3.4.4 Analysis of pH and total soluble solids concentration

Solutions of 1% (w/v) freeze-dried mucilage were used to measure pH with a potentiometer (Orion 420ª, Thermo Fisher Scientific Inc., USA; (Manhivi et al.,

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2018), and total soluble solids concentration (TSSC) was determined, as degrees Brix (°Brix), with a digital refractometer (NR-151, Selecta group, Barcelona, Spain).

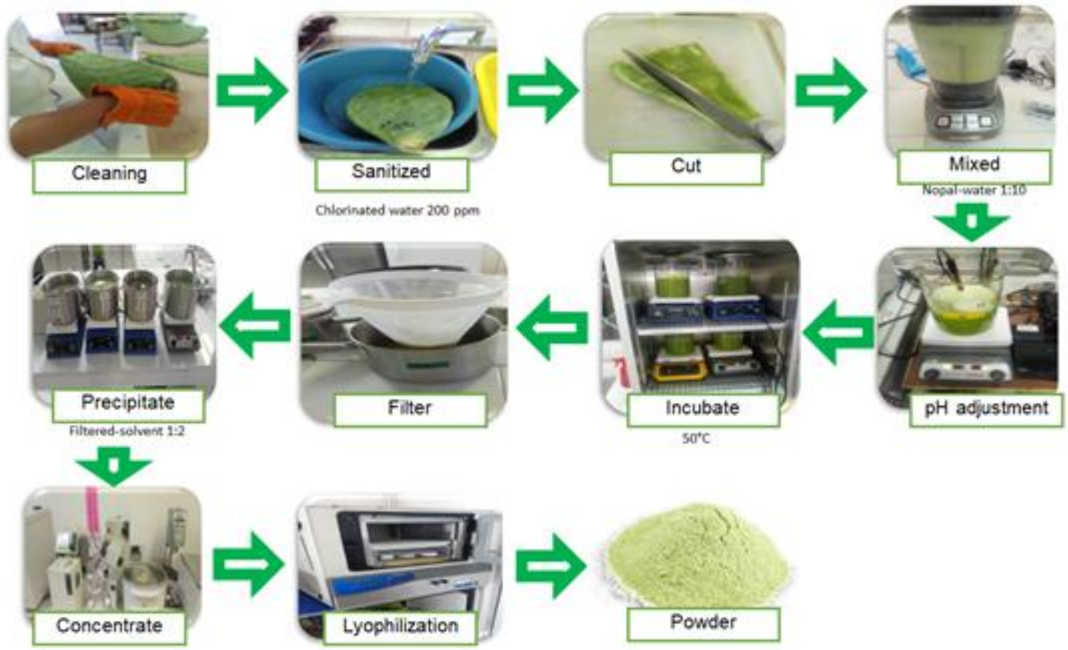

Figure 2. Flow chart of the mucilage extraction process.

3.4.5 Mucilage color

The color of the freeze-dried mucilage was determined with a colorimeter (CR-300 Minolta, Osaka, Japan) using the Hunter Lab color scale. The L* coordinate represents the brightness in a range from 0 (black) to 100 (white). The a* coordinate ranges from green (- a*) to red (+ a*). The b* coordinate ranges from yellow (- b*) to blue (+ b*). The chromaticity (C*) represents the saturation of color and was estimated with the following equation (Eq. 1):

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

The hue angle (°H) indicates the hue of the sample and was calculated with the following equation (Eq 2):

$$°H = tan^{-1}(b^*/a^*) \quad (2)$$

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3.4.6 Chemical composition

The moisture content was determined using the Association of Official Analytical Chemists (AOAC) method 934.06. Bradford´s method was used to determine protein concentration (Bradford, 1976). Ashes content and total dietary fiber was determined according to the methods 923.03 and 985.29 of the AOAC (AOAC, 1990), respectively.

Total carbohydrates

Approximately 500 mg freeze-dried mucilage was mixed with 2.5 mL H_2SO_4 at 72% (w/v) and placed in a water bath for seven min at 50 °C; the acid in the mixture was diluted with distilled water up to 4% (v/v). After that, the solution was left at 121 °C and 9.806 x 10^5 Pa pressure for one h. The resulting sample was filtered through a 40 to 90 µm pore plate crucible at a constant weight. The filtrate was neutralized with $CaCO_3$ and gauged to 75 mL with distilled water. The solutions were paper-filtered (Whatman® Grade 4) and the recovered liquid was filtered through a 0.2 µM membrane. The solutions were stored at -18 °C until analysis (Mussatto-Solange et al., 2011).

Neutral sugars

The determination of neutral sugars was done as described by Soto et al. (2002).

Uronic acid content

The determination of uronic acid content was done as described by Blumenkrantz and Asboe-Hansen (1973). The total carbohydrate content was estimated by adding the neutral sugar content and the uronic acid content.

Monosaccharide analysis

Monosaccharides were determined by liquid chromatography (Agilent 1260 Infinity HPLC, CA, USA) equipped with a refractive index detector set at 45 °C and an Agilent Hi-Plex H 7.7 × 300 mm column at 35 °C (CA, USA). The mobile

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phase was five mM H_2SO_4 at a flow rate of 0.5 mL/min. The injection volume was 20 µL hydrolysates with a retention time of 32 min. The identification and quantification of monosaccharides were performed, respectively, with pure standards (glucose, xylose and arabinose) and previously determined calibration curves.

3.4.7 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy of freeze-dried mucilage samples was carried out in a spectrophotometer (Perkin Elmer FT-IR, Perkin Elmer, Inc., Waltham, MA, USA) in the spectral range of 4000-550 cm^{-1}, performing 16 scans per sample.

3.4.8 Viscosity and molar mass determination

Approximately 500 mg of freeze-dried mucilage was mixed with 20 mL of distilled water and vortexed. The solutions were centrifuged (Sigma 3-18KS, Osterode am Harz, Germany) at 10,000 rpm for 30 min at 25 °C. The supernatant was collected, shaken, and re-centrifuged three times. The solutions were filtered, first through 1.2 µM glass microfiber filters and subsequently through 0.45 µM microfilters. From each mucilage sample, samples at concentrations of six, 12, 15, 15, 18, and 25 mg/mL (w/v) were prepared using distilled water as solvent. Viscosity was measured directly in a viscometer (MicroViscTM, Rheosense Inc., Biolab A/S, Risskov, Denmark) at 25 °C. Three readings per sample were executed and the viscosity was expressed in mPa s.

The intrinsic viscosity (ɳ) was determined using the viscosity of mucilage solutions (from six to 25 mg mL-1; w/v) using the equation (Solomon & Ciută, 1962)

(Eq 3): $$[\eta] = \frac{2(\eta_{sp} - \ln \eta_r)^{1/2}}{C} \quad (3)$$

Where ɳ was already defined, η_{sp} is the specific viscosity, η_r is the relative viscosity, ln is the natural logarithm and C is the sample concentration (g/dL). Likewise, the molar mass was estimated from the intrinsic viscosity using the equation(Wang & Cui, 2005) (Eq 4):

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$$[\eta] = K * M_V{}^{\alpha} \quad (4)$$

where η was already defined, K is the value 3.81 x 10^{-4} dL/g, M_V is the molar mass (g/mol or Da), and α is constant with a dimensionless value of 0.723. The constant values of K and α depend on the polymer shape, the solvent used, and the measurement temperature (Quinzio et al., 2018).

3.4.9 Relative water content

The RWC was determined simultaneously with cladode collection using the following protocol. Tissue samples were obtained randomly from two cladodes of two plants per treatment replicate and deposited in Eppendorf tubes. The tissue samples were individually punched with a punch (17 mm inner Ø). This was done between 12:00 and 13:00 hours. This variable was determined with the equation: CRA = (Mf-Md/Mt-Md) x 100, where Mf, Mt, and Md are the fresh, turgid, and dry mass, respectively.

3.4.10 Data analysis

The information was analyzed in a randomized complete block model with factorial arrangement in the treatments. Fisher's least significant difference test with $p \leq 0.05$ was used in the *post hoc* analysis of treatment means. All calculations were performed in the STATISTICA® 7.0 system (StatSoft, Inc., Tulsa, OK, USA).

3.5 Results and discussion

3.5.1 Mucilage yield

The statistical analysis detected significant interaction among the levels of the factors irrigation and variety for yield, pH, and Brix (Table 3). In general, 'Amarilla Olorosa' and 'Roja Lisa' varieties cultivated under NI produced more mucilage than the other irrigation × variety combinations. The amount of mucilage in cladodes varies according to the genetic load of the variety (López-Palacios et al.,

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2012).

Table 3. Yield, pH, and total soluble solids concentration (TSSC, °Brix) of mucilage from cladodes of *Opuntia* varieties subjected to irrigation regimes.

Main effects/interaction	**Yield (% dry weight)**	**pH**	**TSSC (°Brix)**
Irrigation regime			
Non-irrigated (NI)	17.91 ± 3.14 a[a]	7.65 ± 0.35 b	0.25 ± 0.15 a
Supplemental irrigation (SI)	14.36 ± 1.81 b	7.81 ± 0.31 b	0.16 ± 0.07 b
Full irrigation (FI)	13.76 ± 1.94 b	8.29 ± 0.18 a	0.10 ± 0.00 c
LSD	0.96	0.17	0.04
Significance	0.0000	0.0000	0.0000
Variety			
'Roja Lisa' (RL)	17.11 ± 1.52 a	7.83 ± 0.47 b	0.26 ± 0.16 a
'Cristalina' (C)	13.25 ± 1.54 c	8.06 ± 0.30 a	0.10 ± 0.00 c
'Amarilla Olorosa' (AO)	16.33 ± 4.58 a	7.67 ± 0.43 b	0.20 ± 0.08 b
'Dalia Roja' (DR)	14.68 ± 1.59 b	8.11 ± 0.17 a	0.11 ± 0.04 c
LSD	1.10	0.19	0.05
Significance	0.0000	0.0002	0.0000
Interaction effects			
NI x RL	18.57 ± 0.35 a	7.51 ± 0.29 ef	0.45 ± 0.07 a
NI x C	14.45 ± 1.76 de	7.85 ± 0.13 cd	0.10 ± 0.00 d
NI x AO	22.22 ± 0.49 a	7.22 ± 0.03 f	0.30 ± 0.00 b
NI x DR	16.38 ± 0.91 c	8.03 ± 0.02 bc	0.15 ± 0.00 c
SI x RL	16.65 ± 1.50 bc	7.56 ± 0.20 de	0.25 ± 0.07 b
SI x C	13.12 ± 1.46 ef	8.05 ± 0.26 bc	0.10 ± 0.00 d
SI x AO	14.46 ± 0.39 de	7.58 ± 0.05 de	0.20 ± 0.00 c
SI x DR	13.22 ± 1.16 ef	8.06 ± 0.26 bc	0.10 ± 0.10 d
FI x RL	16.12 ± 1.38 cd	8.42 ± 0.04 a	0.10 ± 0.00 d
FI x C	12.18 ± 0.63 f	8.29 ± 0.35 ab	0.10 ± 0.00 d
FI x AO	12.31 ± 1.50 f	8.20 ± 0.10 ab	0.10 ± 0.00 d
FI x DR	14.43 ± 0.56 de	8.26 ± 0.09 ab	0.10 ± 0.00 d
LSD	1.92	0.334	0.007
Significance	0.0000	0.0199	0.0633

[a]Within each column, mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to Fisher's least significant difference (LSD) test at $p \leq 0.05$.

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However, in addition to genetic load, the main effect of irrigation suggests that mucilage yield and total soluble solids concentration (TSSC, °Brix) may have been associated with a dilution phenomenon (Zegbe-Domínguez et al., 2003), measured here as relative water content (RWC), since as the amount of water increased, mucilage yield (r = -0.54; p = 0.001) and TSSC (r = -0.40; p = 0.055) decreased (Table 3). As water stress increases, the amount of mucilage in the hydrenchyma increases (Zaferanieh & Mahdavi, 2021). This is possible because mucilage compounds, along with the solutes, retain more water, and thus, increase the plant's resistance to water deficit (Nobel et al., 1992).
The difference in mucilage yield between SI and NI plants averaged ~3%, but when SI was applied, annual fresh biomass increased up to 10-fold in these plants, suggesting greater mucilage yield in SI plants in a commercial-scale scenario (Neupane et al., 2021). Mucilage pH was more alkaline in all cactus varieties with FI and decreased proportionally in all varieties in SI and NI plants (Table 3). This could, in part, be associated with the presence of uronic acids in mucilage, since low pH values (less alkaline) are inversely related (r = -0.08; p = 0.70) to the uronic acid content (Procacci et al., 2021) (Table 4). Although this was not measured, the increase in pH could also be associated, in part, with increased salts in mucilaginous cells, which were absorbed and transported as water content increased in the cladodes of FI and SI plants (r = 0.29; p = 0.09) (Pandey, 2015).

3.5.2 Mucilage color

The average values of the color parameters L* (82.35), a* (-2.55), b* (15.94), C* (16.15), and °H (98.89) indicate that the mucilage samples were off-white powders with yellow-green tints. These values are consistent with previous reports (Dick et al., 2019).
There was no significant interaction between the irrigation levels and variety in any color attributes (Table 4). NI plants produced, on average, a mucilage powder

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with higher L*, less green (a*), and yellow (b*) than mucilage powder from plants receiving SI or FI.

The latter was corroborated by the increase in C* and °H in mucilage powder from plants that received SI or FI (Table 4). The latter behavior may be associated with high ash contents in the mucilage samples (Teterycz et al., 2020), which coincided with the high ash concentration in mucilage powder in plants undergoing SI or FI (Table 5).

Table 4. Color parameters of mucilage from cladodes of *Opuntia* varieties subjected to irrigation regimes.

	Color parameters[a]				
Main effects	**L***	**a***	**b***	**C***	**°H**
Irrigation regime					
Non-irrigated	84.13 ± 3.09 a[b]	-2.00 ± 1.06 a	14.33 ± 3.53 b	14.93 ± 4.81 b	97.70 ± 2.44 b
Supplemental irrigation	82.40 ± 3.50 b	-2.69 ± 1.06 b	16.50 ± 3.65 a	16.00 ± 3.88 b	99.50 ± 2.28 a
Full irrigation	80.92 ± 4.19 c	-2.86 ± 0.91 b	17.08 ± 3.24 a	17.94 ± 3.53 a	99.20 ± 1.56 a
LSD	1.46	0.24	1.6	1.11	1.05
Significance	0.0005	0.0000	0.0000	0.0000	0.0228
Variety					
‘Roja Lisa’	85.86 ± 2.26 a	-1.53 ± 0.52 a	11.59 ± 1.51 c	10.81 ± 2.31 d	97.45 ± 2.03 c
‘Cristalina’	81.13 ± 1.93 c	-2.62 ± 0.52 c	18.38 ± 1.35 a	18.40 ± 1.32 b	98.06 ± 1.64 bc
‘Amarilla Olorosa’	83.98 ± 2.60 b	-2.07 ± 0.32 b	14.20 ± 1.85 b	14.56 ± 2.13 c	98.72 ± 1.79 b
‘Dalia Roja’	78.70 ± 3.30 d	-4.07 ± 0.39 d	19.29 ± 2.29 a	20.76 ± 2.04 a	101.50 ± 0.78 a
LSD	1.7	0.28	1.8	1.3	1.21
Significance	0.0000	0.0000	0.0000	0.0000	0.0000

[a]L* = luminosity, a*= red (+) or green (-) axis, b*= yellow (+) or blue (-) axis, C*= chromaticity, °H= Hue angle.
[b]Within each column, mean values (± standard deviation; n = 3) with different letters indicate statistical differences according to Fisher's least significant difference (LSD) test at $p \leq 0.05$.

The main effect of variety was that, on average, mucilage powder from 'Roja Lisa' plants had the highest L*, with lower values of a* and b* (less green and yellow) than the other varieties included in this study. The above was consistent with the average values of C* and °H for mucilage powder from 'Roja Lisa' plants.

Therefore, mucilage powder from this variety can produce solutions with colorations that, in theory, will not interfere with the color of the product when used

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as an additive (Du Toit et al., 2019). The genetic component of color properties differed significantly among varieties, which had not been documented previously. Such properties can be exploited by the industry.

3.5.3 Chemical composition

There was no significant interaction between irrigation level and the variety on the chemical composition of mucilage. The moisture and ash contents of mucilage from plants receiving FI were, on average, greater than in the mucilage from plants receiving SI or NI (Table 5). The increased moisture and ash (minerals, primarily Mg, P, K, and Ca) can be explained, in part, by a direct effect of increased water absorbed by the root systems of plants receiving FI. The opposite occurs in temperate fruit trees exposed to water deficit (Casamali et al., 2021) or induced stress due to salts (Liu et al., 2020). In this study, the association between RWC and moisture content was low (r = 0.47; p = 0.02) and very low with ash content (r = 0.19; p = 0.36). In contrast, NI plants produced, on average, the most protein, total fiber, and total carbohydrates (Table 5). The increased protein in these plants may be associated with the *de novo* synthesis of protective proteins triggered by abiotic stress (Yang et al., 2021), which may occur in NI plants. The high concentration of carbohydrates (stored energy) in NI plants may be associated with a lack of growth in sink tissues (Slewinski & Braun, 2010) due to the water deficit imposed on NI plants. Cactus pear cladodes collected in the dry season also had more carbohydrates (Ribeiro et al., 2010), which can be used potentially as an energy source.

The difference in the chemical composition of the cladodes is mainly attributed to their genetic load (Mokoboki & Sebola, 2017; Muñoz de Chávez et al., 1995). The latter was confirmed by the analysis of the main effect of varieties (Table 5). The mucilage (on a dry weight basis) of 'Dalia Roja' plants, on average, had greater percent moisture, protein, and ash, but also presented the least total fiber and carbohydrates of the tested varieties (Table 5). Compared with other studies on

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cladode chemical composition (Mokoboki & Sebola, 2017; Muñoz de Chávez et al., 1995), the mucilage protein content of our four cactus varieties was low, because it was determined only in mucilaginous cells, which are known to have little or no protein (Trachtenberg & Mayer, 1981). However, the low mucilage protein content, here observed, shows the efficacy of our extraction protocol, because high protein contents would show polysaccharide contamination (Bayar et al., 2016). In general, there was more carbohydrate in the mucilage of NI plants, while the SI and FI treatments had similar amounts ($p \leq 0.05$).

Table 5. Chemical composition (% dry matter) of mucilage extracted from cladodes of *Opuntia* varieties subjected to irrigation regimes.

Main effects	**Moisture (%)**	**Protein**	**Ashes**	**Total fiber**	**Total carbohydrates**
Non-irrigated	4.07 ± 0.41 c[a]	0.92 ± 0.30 a	14.30 ± 3.26 c	65.80 ± 4.11 a	75.45 ± 6.47 a
Supplemental irrigation	4.66 ± 0.50 b	0.81 ± 0.23 b	16.09 ± 3.71 b	61.88 ± 4.42 b	69.96 ± 8.70 b
Full irrigation	5.69 ± 0.21 a	0.69 ± 0.22 c	18.03 ± 4.36 a	59.81 ± 5.27 c	68.64 ± 7.28 b
LSD	0.36	0.06	0.87	1.30	3.2
Significance	0.0000	0.0000	0.0000	0.0014	0.0000
Varieties					
'Roja Lisa'	4.92 ± 0.56 ab	0.44 ± 0.06 d	11.13 ± 1.29 c	68.57 ± 2.14 a	78.10 ± 3.90 a
'Cristalina'	4.69 ± 0.75 bc	0.96 ± 0.18 b	19.46 ± 1.98 a	60.20 ± 4.04 c	64.58 ± 3.53 b
'Amarilla Olorosa'	4.49 ± 0.99 c	0.73 ± 0.08 c	14.69 ± 1.22 b	63.51 ± 2.86 b	77.97 ± 3.60 a
'Dalia Roja'	5.12 ± 0.80 a	1.04 ± 0.13 a	19.28 ± 2.68 a	57.72 ± 3.36 d	64.76 ± 5.07 b
LSD	0.42	0.08	1.00	1.50	3.67
Significance	0.0320	0.0000	0.0000	0.0000	0.0014

[a]Within each column, mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to Fisher's least significant difference (LSD) test at $p \leq 0.05$.

This is consistent with previous findings that cactus pear cladodes collected during the dry season had more carbohydrates than those collected during the rainy season (Ribeiro et al., 2010). Little plant growth is observed during the dry season; therefore, cladodes are serving as carbohydrates storage. In this study, the association of RWC with fiber (r = -0.24; $p = 0.25$) and total carbohydrate (r = -0.24; $p = 0.26$) content was low, which deserves further study.

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Sugar and uronic acid composition

Monosaccharide analysis revealed the presence of glucose (29.98 to 61.36 %), xylose (11.77 to 42.99 %), arabinose (13.51 to 27.02 %), and uronic acids (4.30 to 9.32 %) in the mucilages of the four cactus pear varieties (Table 6). The latter suggests that the structure of the mucilage is a xyloglucan skeleton of the XXGG type (Xyl, Xyl, Glc, Glc) with arabinose branches joined to xylose residues. However, the branching pattern (side chains) of xyloglucans may change depending on the variety (York, 2004), as suggested in this study.

The statistical analysis detected an interaction between irrigation level and variety for xylose and arabinose content only. According to the main effect of irrigation, NI plants had the most glucose and uronic acids (Table 6). This was consistent with findings in other cactus cladodes collected in the dry season, which had more uronic acid (Ribeiro et al., 2010). This is explained, in part, by the association of RWC with uronic acid concentration ($r = -0.52$; $p = 0.01$): because as soil water content was greater in SI and FI plants, uronic acid content decreased. The glucose content behaved similarly to uronic acid content; however, the association with RWC was weak and not significant ($r = -0.26$; $p = 0.22$). As glucose is the basic substrate for the synthesis of starch, cellulose, sucrose, and other carbohydrates, its reduction in SI and FI plants could be associated with catabolism toward simpler compounds needed at points of demand (Atwell et al., 1999). Among varieties, 'Roja Lisa' mucilage had the most glucose, while 'Cristalina' mucilage had the most arabinose, xylose, and uronic acids. These differences could be explained by epi and/or genetic differences among these *Opuntia* species (Cruz-Rubio et al., 2020).

Xylose and arabinose are the main monosaccharide components of the primary cell wall, considered important in the linkage between pectic polysaccharides, hemicellulose, and cellulose (Li et al., 2015).

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Table 6. Concentrations of sugars and uronic acids (% molar) in mucilage from cladodes of *Opuntia* varieties subjected to irrigation regimes.

Main effects/interaction	Glucose	Xylose	Arabinose	Uronic acids
Irrigation system				
Non-irrigated (NI)	52.00 ± 8.68 a[a]	16.52 ± 6.80 c	23.26 ± 3.08 a	8.19 ± 1.02 a
Supplemental irrigation (SI)	47.57 ± 9.85 b	25.59 ± 7.45 b	20.38 ± 4.24 b	6.44 ± 1.21 b
Full irrigation (FI)	45.42 ± 11.03 c	30.87 ± 8.67 a	17.31 ± 3.02 c	6.38 ± 1.77 b
LSD	1.6	1.30	1.3	0.78
Significance	0.0000	0.0000	0.0000	0.0004
Variety				
'Roja Lisa' (RL)	60.08 ± 1.42 a	17.54 ± 4.65 d	15.60 ± 3.01 b	6.76 ± 0.57 b
'Cristalina' (C)	34.42 ± 4.18 c	35.78 ± 7.12 a	22.35 ± 1.71 a	7.44 ± 1.60 b
'Amarilla Olorosa' (AO)	49.19 ± 3.25 b	23.54 ± 9.03 b	21.78 ± 4.95 a	5.47 ± 1.55 c
'Dalia Roja' (DR)	49.64 ± 4.13 b	20.45 ± 5.56 c	21.53 ± 2.69 a	8.36 ± 0.82 a
LSD	1.83	1.46	1.47	0.91
Significance	0.0000	0.0000	0.0000	0.0001
Interaction effects				
NI x RL	61.36 ± 1.04 a	11.77 ± 0.43 f	19.39 ± 0.76 de	7.48 ± 0.15 a
NI x C	39.08 ± 1.00 a	27.32 ± 1.73 d	24.28 ± 0.06 b	9.32 ± 0.79 a
NI x AO	52.93 ± 1.00 a	12.78 ± 0.77 f	27.02 ± 0.06 a	7.27 ± 0.73 a
NI x DR	54.66 ± 0.48 a	14.25 ± 1.95 f	22.36 ± 2.04 bc	8.72 ± 0.57 a
SI x RL	60.14 ± 0.54 a	19.58 ± 1.86 e	13.91 ± 1.32 gh	6.36 ± 0.00 a
SI x C	34.21 ± 0.25 a	37.03 ± 0.01 b	21.85 ± 0.74 bcd	6.89 ± 1.00 a
SI x AO	47.84 ± 0.25 a	25.13 ± 1.02 d	22.18 ± 1.85 bc	4.84 ± 0.80 a
SI x DR	48.12 ± 2.33 a	20.62 ± 0.10 e	23.56 ± 2.43 b	7.70 ± 0.00 a
FI x RL	58.74 ± 1.39 a	21.29 ± 1.31 e	13.51 ± 0.12 h	6.45 ± 0.20 a
FI x C	29.98 ± 1.88 a	42.99 ± 0.71 a	20.91 ± 1.43 cde	6.12 ± 0.26 a
FI x AO	46.82 ± 1.88 a	32.72 ± 1.29 c	16.15 ± 0.94 fg	4.30 ± 0.94 a
FI x DR	46.82 ± 1.88 a	26.51 ± 0.90 d	18.66 ± 0.20 ef	8.67 ± 1.32 a
LSD	3.2	2.53	2.56	1.57
Significance	0.0846	0.0013	0.0026	0.1098

[a]Within each column, mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to Fisher's least significant difference (LSD) test at $p \leq 0.05$.

However, the concentration of these sugars depends, among other factors, on the phenological stage of the cladode (Vargas-Solano et al., 2022) and water

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availability in the soil profile (Ribeiro et al., 2010).

It is probable that in cactus pear, drought activates enzymes involved in monosaccharide synthesis, leading to changes in polysaccharide composition (Galloway et al., 2020). The cladodes used in this study were mature and reproductive (> 1 year) and their molar concentrations were associated with RWC (xylose, $r = 0.56$; $p = 0.004$ and arabinose, $r = -0.46$; $p = 0.022$). The interaction, for these monosaccharides, was a function of genetic load among varieties (Table 6).

The composition, structure, and conformational diversity of the polysaccharide are responsible for some remarkable characteristics of mucilages such as water-holding capacity, emulsification capacity, and rheological properties (De Andrade-Vieira et al., 2021; Dickinson, 2018; Wu et al., 2009). The composition and structure of *Opuntia* heteropolysaccharides, such as mucilage, are related to their availability as a carbon source and their prebiotic potential (Cruz-Rubio et al., 2020). Thus, mucilage properties are fundamental to their use in the agroindustry.

3.5.4 Fourier Transform Infrared Spectroscopy

The FTIR of all mucilages showed signals characteristic of a polysaccharide (Figure 3). The bands in the region between 3500 and 3200 cm^{-1} indicate the presence of hydroxyl groups (-OH) related to the intermolecular bonds of alcohols and carboxylic acids, while the absorption bands between 3000 - 1750 cm^{-1} are representative of the -CH and CH_2 radicals. The absorption bands between 2920 and 2830 cm^{-1} are attributed to the stretching vibration of the CH radical. These absorption bands are attributed to functional groups of neutral polysaccharide components of mucilage, such as arabinose and xylose. The bands in the region between 1800 and 1500 cm^{-1} indicate the C=C and C=O vibration bonds. The band at 1600 cm^{-1} corresponds to the stretching vibration of the C=C bonds of the carboxyl group (-COOH).

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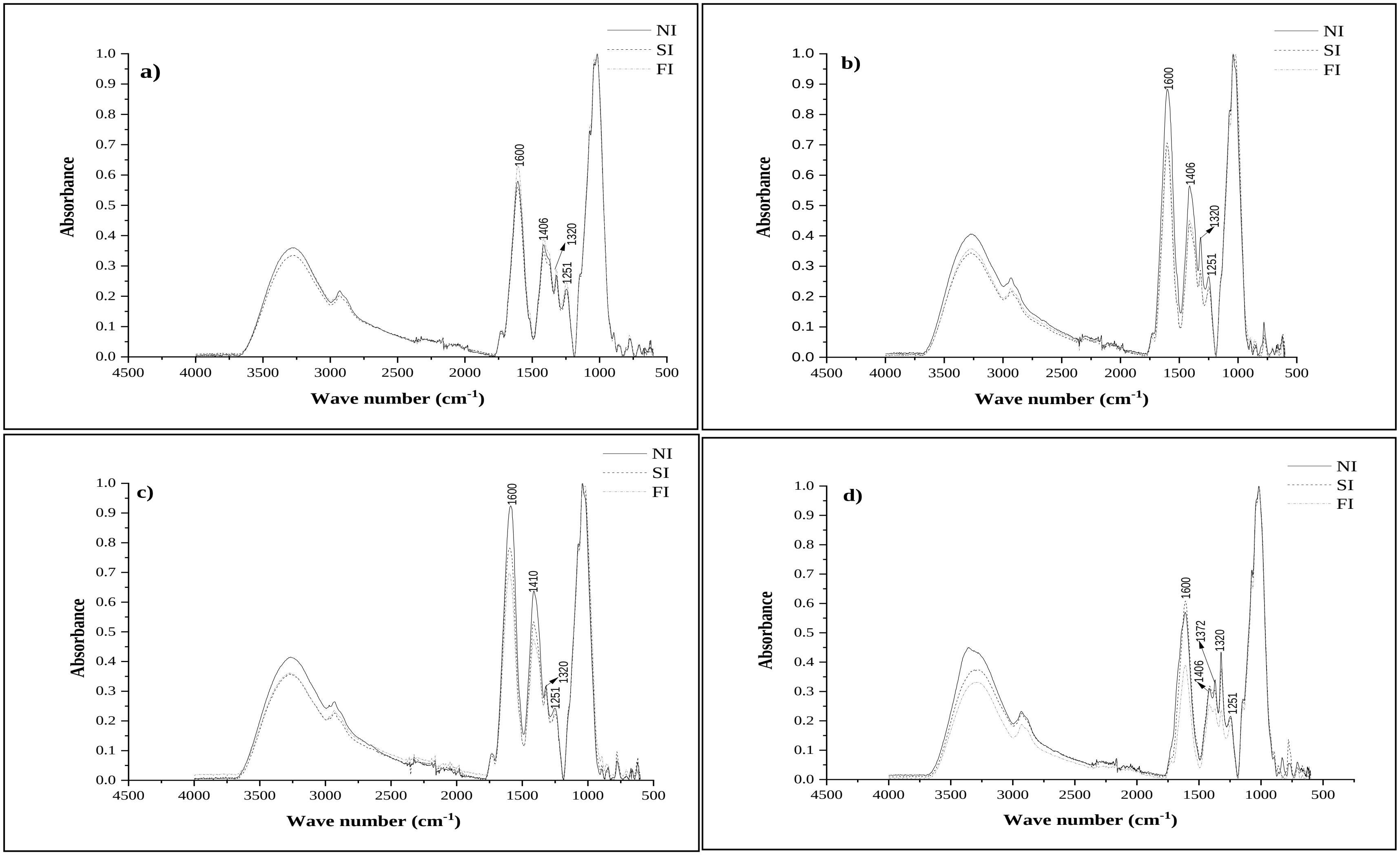

Figure 3. Fourier-Transform Infrared spectra of cladode of freeze-dried mucilage of 'Amarilla Olorosa' (a), 'Cristalina' (b), 'Dalia Roja' (c), and 'Roja Lisa' (d) cactus pear varieties, subjected to three irrigation regimes: non-irrigated (NI, control), supplemental irrigation (SI) and full irrigation (FI)

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The absorption band between 1700 and 1600 cm^{-1} indicates the stretching of the C=O bond of the amide group, indicating the presence of proteins. The absorption band at 1407 cm^{-1} probably corresponds to the -OH radical of polyphenolic compounds. The absorption bands between 1320 and 1210 cm^{-1} correspond to the stretching of the C-O bond of carboxylic acids and indicate the presence of uronic acid. The absorption band between 1085 and 1045 cm^{-1} is attributed to monosaccharides such as glucose and the absorption band at 895 cm^{-1} corresponds to anomeric β-carbon, indicating the presence of β-type glycosidic bonds in mucilages (Bernardino-Nicanor et al., 2018).

The mucilage spectra of plants that were not irrigated had more intense bands, indicating higher contents of carboxylic acids, proteins, and uronic acids. This is consistent with our findings of increased proteins, some monosaccharides, and uronic acids in mucilage from NI plants. This suggests that these compounds are synthesized by cactus pear plants as a possible defense mechanism against drought, either for water retention or energy storage. In response to a severe drought, maize plants showed changes in the infrared bands corresponding to proteins and carbohydrates (Ogbaga et al., 2017).

3.5.5 Viscosity and molar mass

Viscosity measures a substance's resistance to shear or tensile stresses. Cladode mucilage viscosity was greater in cactus pear plants grown in low-rainfall areas (Sáenz et al., 2004). We found association of RWC with mucilage viscosity ($r = -0.65$; $p = 0.001$) and molecular weight ($r = -0.77$; $p = 0.000$). The interaction between irrigation level and variety confirmed these associations (Figures 4 and 5). The viscosity of the mucilage solutions was 192.91 % greater in NI plants. The mucilage solutions of 'Amarilla Olorosa' cladodes from NI plants showed the greatest viscosity and the viscosity decreased proportionally when plants were exposed to SI and FI. This decrease with irrigation was consistent across varieties

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(Fig. 2). The average mucilage viscosity of 'Amarilla Olorosa' suggests that this variety is very sensitive to water supply, while the mucilage viscosity of 'Roja Lisa' and 'Cristalina' was similar in plants with SI or FI. In contrast, the average mucilage viscosity of 'Dalia Roja' varied markedly due to irrigation treatment (Figure 4). Interestingly, the mucilage from all NI treatments had the most uronic acids and arabinose for their variety. This may be related to the increased viscosity, since the carboxyl groups of uronic acids can interact with water molecules or with certain cations, such as calcium, increasing the viscosity of the solution (Procacci et al., 2021; Rodríguez-González et al., 2014). Also, mucilage with more arabinose (as detected in all SI treatments) can generate suspensions with greater viscosity because its functional groups are more willing to interact intermolecularly (Rodríguez-González et al., 2014).

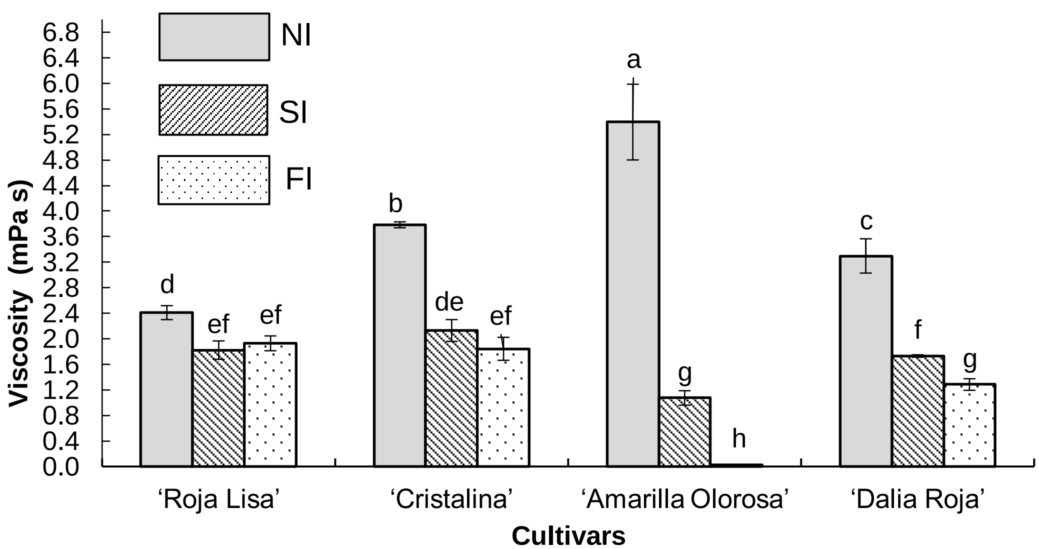

Figure 4. Viscosity (mPa s) of mucilage from *Opuntia* varieties subjected to three irrigation regimes: no irrigation (NI, control), supplemental irrigation (SI), and full irrigation (FI). Mean values (± standard deviation; n = 3) with different letters indicate statistical differences ($p \leq 0.05$) according to the Fisher's least significant difference (LSD) test at $p \leq 0.05$. Viscosity measurements were obtained at 25 °C with distilled water as the solvent.

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The molar mass of mucilage ranged from 58 to 214 kDa. The significant interaction between irrigation level and variety followed the same pattern observed for viscosity (Figures 4 and 5). The greater molar mass of NI mucilages may reflect differences in their composition, structure, and number of functional groups. These differences are important because the molar mass of polysaccharides can affect rheological behavior (Janjarasskul & Krochta, 2010) and biological activities, including antioxidant, hypolipidemic, antiviral (Wang et al., 2022), and prebiotic potential (Cruz-Rubio et al., 2020).

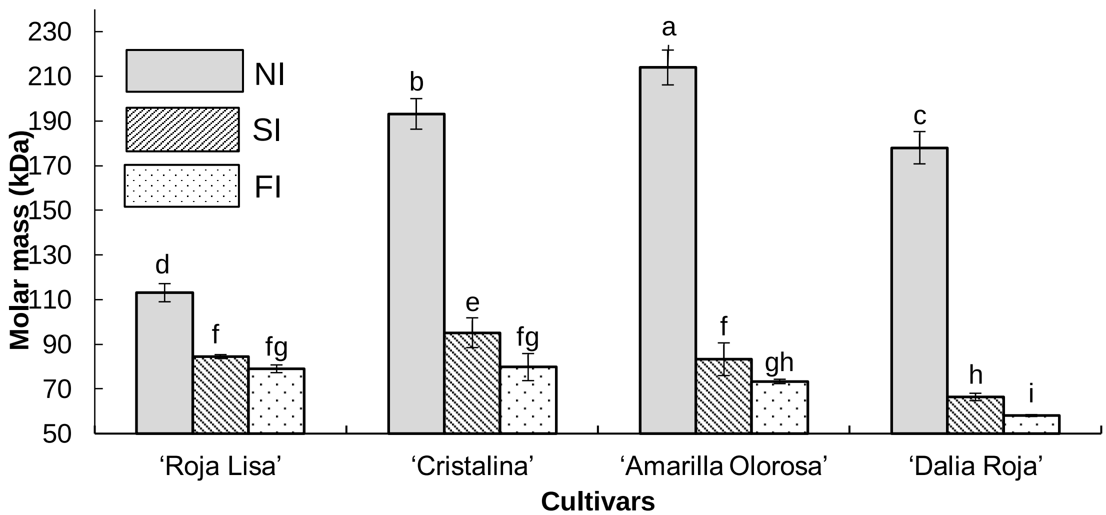

Figure 5. Molar mass (g/mol) of mucilage from *Opuntia* varieties subjected to three irrigation regimens: no irrigation (NI), supplemental irrigation (SI), and full irrigation (FI). Mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to the Fisher's least significant difference (LSD) test at $p \leq 0.05$.

For example, high molecular weight polysaccharides form many intermolecular associations, creating greater cohesive strength and increasing the thickening effects and viscosity of the solution (Janjarasskul & Krochta, 2010). These polysaccharides could form gels and protect intestinal cells when consumed (Huo

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et al., 2022). Low molar mass polysaccharides have greater antioxidant activity because they contain more free hydroxyl groups to accept and scavenge hydrogen radicals (Jia et al., 2021).

3.6 Conclusions

We hypothesized that irrigation would modify some physicochemical characteristics of mucilage extracted from cladodes of different cactus pear varieties. Statistical evidence did not reject this hypothesis because, regardless of the cactus pear variety, irrigation modified both mucilage yield, composition, and physicochemical properties studied here. Also, the mucilage of non-irrigated plants was more luminous, with less green tone and yellow tone than mucilage from plants that received supplemental or full irrigation. Furthermore, mucilage from non-irrigated plants not only had the least ash, but also more protein, total fiber, total carbohydrates, glucose, arabinose, and uronic acids, greater viscosity, and increased molar mass.

The genetic load among varieties influenced the physicochemical characteristics of the mucilage. 'Amarilla Olorosa' and 'Roja Lisa' plants receiving no irrigation produced more mucilage than ‘Cristalina’ and ‘Dalia Roja’ plants. Mucilage from 'Roja Lisa' cladodes had the highest L* and lower a* and b* shades than the other varieties. The mucilage from 'Amarilla Olorosa' plants with no irrigation was the most viscous. Mucilage from 'Dalia Roja' plants had the most protein and ash, but the least total fiber and total carbohydrate.

Restricting the amount of water to cactus pear varieties studied here could be a feasible strategy to obtain high-quality mucilage in regions with low rainfall and limited water availability for irrigation. In supplementally irrigated cactus pear orchards, cladodes can be collected at pruning, because irrigation is suspended at fruit harvest and cladode yield can be almost 3-fold higher than non-irrigated plants, and therefore, mucilage yield increases. The mucilage characteristics

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observed here suggest a potential use not only for the food and pharmaceutical industry much more diversified but also for other industrial applications.

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4. FUNCTIONAL PROPERTIES, BIOACTIVE COMPOUNDS, AND ANTIOXIDANT CAPACITY OF *Opuntia* MUCILAGE UNDER IRRIGATION REGIMES AND ITS USE AS AN EDIBLE FILM

4.1 Resumen

El mucílago de *Opuntia* spp. es un compuesto con diversas aplicaciones en la industria alimentaria. Sin embargo, el riego a plantas de nopal podría modificar las propiedades funcionales y capacidad antioxidante de este hidrocoloide motivo de esta investigación. En este estudio se probaron tres regímenes de riego: sin riego (SR), riego suplementario (RS), donde la capacidad campo (CC = 0.28 m^3 m^{-3}) se restableció cuando el contenido de agua en el suelo estuvo cerca o igual al punto de marchitez permanente (PMP = 0.14 m^3 m^{-3}) y riego completo (RC) donde el 100 % de la evapotranspiración del cultivo fue aplicado en cuatro variedades de nopal tunero 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' y 'Roja Lisa'. Muestras de cladodios fueron recolectadas para la extracción de mucílago por maceración. Las propiedades funcionales, contenido de polifenoles totales, perfil de polifenoles y capacidad antioxidante fueron analizadas en el mucílago. En general, el polvo de mucílago de las plantas variedad 'Dalia Roja' bajo RC presentó los mayores valores de capacidad de retención de aceite (CRAc), pero tuvo los valores más bajos en índice de hinchamiento, solubilidad y capacidad de retención de agua (CRA). El mucílago de la variedad 'Roja Lisa' cultivada SR exhibió la mayor solubilidad y CRA. Por otra parte, el mucílago de plantas de la variedad 'Roja Lisa' con RC produjo los menores valores de contenido polifenólico total y capacidad antioxidante, mientras que el mucílago de la combinación 'Cristalina' cultivada SR produjo los mayores valores de índice de hinchamiento, contenido polifenólico total y capacidad antioxidante. Debido a dichas características, el mucílago de la variedad 'Cristalina' con SR se usó en combinación con alginato de sodio para la elaboración de un recubrimiento comestible, el cual se aplicó a mitades de aguacate. Este recubrimiento fue efectivo para conservar la calidad comercial de mitades de aguacate. Por lo tanto, las plantas de nopal de las variedades incluidas en este estudio bajo el sistema SR, podría ser una estrategia factible para producir mucílago con características sobresalientes para la formulación de recubrimientos comestibles para aguacate mínimamente procesado.

Palabras clave: Cladodios, cubierta comestible, mitades de aguacate fresco.

4.2 Abstract

The mucilage of *Opuntia* spp is a compound with diverse applications in the food industry. However, irrigation of nopal plants could modify the functional properties and antioxidant capacity of this hydrocolloid. In this study, three irrigation regimes were tested: Non-Irrigated (NI), Supplemental Irrigation (SI) (FC 0.28 m^3m^{-3} and PWP 0.14 m^3 m^{-3}), and Full Irrigation (FI) (100 % of crop evapotranspiration), on four cactus varieties 'Amarilla Olorosa', 'Cristalina', 'Dalia Roja' and 'Roja Lisa'. Cladode samples were collected for mucilage extraction by maceration. The functional properties, total polyphenol content, polyphenol profile, and antioxidant capacity were analyzed in the mucilage. In general, the mucilage powder from 'Dalia Roja' FI plants showed the highest oil holding capacity (OHC) values, but the lowest values for swelling index, solubility, and water retention curve (WRC). The mucilage of the 'Roja Lisa' plants under SR exhibited the highest solubility and WRC. Besides, mucilage from 'Roja Lisa' plants under FI had the lowest total polyphenolic content and antioxidant capacity values, while mucilage from the 'Cristalina' plants under NI produced the highest swelling index, total polyphenolic content, and antioxidant capacity values. Based on these characteristics, the mucilage of the 'Cristalina' plants under NI was used, mixing it with sodium alginate for edible film preparation. This coating was effective in maintaining the commercial quality of avocado halves. Therefore, limiting water to cactus plants of the varieties studied here could be a feasible strategy to produce mucilage with remarkable characteristics to elaborate edible coatings for fruits and vegetables.

Keywords: Cladodes, edible coating, fresh avocado halves.

4.3 Introduction

The mucilage extracted from *Opuntia* is a linear heteropolysaccharide that forms a hydrocolloid (Caldera-Villalobos et al., 2022), it can be found in specialized storage cells or free within cells or intracellular spaces of chlorenchyma and parenchyma tissue of cladodes (Sáenz et al., 2004). The mucilage is synthesized in plants for water retention purposes through the secretion of polysaccharides in the extracellular spaces (Archana et al., 2013) during episodes of moisture deficiency in the soil (Nobel & Bobich, 2002). In addition, it plays an important role in the maintenance of ionic balance in plant cells, frost tolerance, water transport, wound response in plant tissue, during plant-host-pathogen interaction, and carbohydrate reserves (Bhurat et al., 2011). This hydrocolloid exhibits molecular masses between 2.3×10^4 and 4.3×10^6 Da (Medina-Torres et al., 2000) and is composed of L-arabinose, D-galactose, D-xylose, L-rhamnose, glucuronic acid and D-galacturonic acid (Garfias Silva et al., 2022).

Opuntia mucilage presents foaming and emulsifying properties, water retention capacity, oil retention capacity, and viscosity modification, as well as the excellent antioxidant potential that allows it to be a compound with notable prospects as an additive for the food industry (Bayar et al., 2016). For this reason, mucilage has been widely used as a thickening agent (Carpintero-Tepole et al., 2021), fat substitute (Bernardino-Nicanor et al., 2015), stabilizer (Du Toit et al., 2019), bioactive compound encapsulation material (Soto-Castro et al., 2019) and as a coating for fresh or minimally processed fruits and vegetables (Liguori et al., 2021).

In addition, it has been documented that the mucilage content of the nopal cladode changes with crop management, environmental temperature, and soil water content (Goldstein et al., 1991; Sáenz et al., 2004). Likewise, the chemical composition and physicochemical properties of mucilage can be modified by plant species, cladode age, and month of harvest (Contreras-Padilla et al., 2016; Messina et al., 2021; Rodríguez-González et al., 2014).

On the other hand, in rain-fed crops such as prickly pear cactus, several agronomic practices have been implemented such as the SI (Zegbe & Palestina, 2020). The SI is a strategy to maintain usable moisture in the soil and increase water productivity and crop yields. Neupane et al. (2021) demonstrated an increase in cactus biomass production under SI conditions. Likewise, this irrigation system improved the yield, the size (Arba et al., 2018; Van Der Merwe et al., 1997), preserved pulp firmness, and increased the shelf life of prickly pear cactus fruit (Zegbe, 2015; Zegbe & Palestina, 2020). Also, in grasses such as wheat, it has been documented that SI can increase yield without having a significant nutritional impact on antioxidant and mineral micronutrient concentrations (Wang et al., 2021b). While, in hazelnuts, the antioxidant capacity increased with the application of SI (Tonkaz et al., 2020). However, the effect of water supply through irrigation, during the period of no or little rainfall, on the functional properties and antioxidant capacity of *Opuntia* mucilage has not been evaluated. Therefore, the objective of this study was to evaluate the effect of irrigation on the functional properties, total phenolic content, and antioxidant capacity of mucilage extracted from cladodes of different varieties of prickly pear cactus. As well as to use the mucilage in conjunction with sodium alginate for the elaboration of an edible coating and to increase the shelf life of minimally processed avocado.

4.4 Material and methods

4.4.1 Plant material and experimental process

See section 3.4.2 (pages 53 and 54).

4.4.2 Mucilage extraction

See section 3.4.3 (page 54).

4.4.3 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), vortexed for 1 min, and the volume occupied was recorded. The suspensions were shaken and allowed to settle at room temperature for 16 h (Deeksha et al., 2014). Subsequently, the swelling index with the following equation:

$$Swelling\ Index\ (\%) = \frac{Final\ volume - Initial\ volume}{Final\ volume} x100 \quad (5)$$

Water retention capacity (WRC)

The WRC was determined according to the method proposed by Femenia et al. (1997). Exactly 0.15 g of lyophilized mucilage in 5 mL of sodium phosphate buffer (1 M, pH 6) was vortexed for 1 min and allowed to settle for 24 h at room temperature. Then, the solutions were centrifuged at 6000 rpm for 15 min. The supernatant was filtered through glass fiber (GF/C), recovering the solids, and mixed with the sediment. Finally, the obtained solids were weighed (W_1) and dried at 100 ± 2 °C overnight. Subsequently, the dry weight (W_2) was determined and the WRC was calculated using the following equation:

$$WRC = \frac{W_1 - W_2}{W_2 - k} \tag{6}$$

Where k = α(W_1−W_2) with α = 0.028 g phosphate/mL. WRC was expressed as g H_2O/g mucilage.

Oil holding capacity (OHC)

The mucilage samples (0.15 g) were mixed with 5 mL of sunflower oil and vortexed for 1 min. Then, the suspensions were left to rest for 12 h at room temperature and later, they were centrifuged at 6000 rpm for 10 min. The supernatant was removed and the solids were weighed. OHC was expressed as g oil/g of mucilage (Minjares-Fuentes et al., 2017).

Solubility

The solubility test was carried out according to Dick et al. (2019) with some modifications. Approximately 0.25 g of lyophilized mucilage was weighed and 10 mL of distilled water (60 °C) was added. The mixture was vortexed and homogenized in Ultraturrax IKA T25D (IKA® Works, Inc, Wilmington, USA) at 13,500 rpm for 1 min. The samples were shaken by magnetic stirring for 2 h (60 °C) to complete their hydration. Afterward, the solutions were centrifuged at 6000 rpm for 20 min and the supernatant was filtered on glass fiber (GF/C). The precipitate was oven-dried at 100 °C for 24 h and weighed. The percent of solubility was calculated using the following equation:

$$Solubility\,\% = \frac{W_1 - W_2}{W_1} * 100 \tag{7}$$

Where W_1 is the flesh weight of the sample and W_2 is the weight of the dry sample.

4.4.4 Total polyphenols

Total phenolic content was measured using a modification of the Folin-Ciocalteau method. Solutions of 10 mg of lyophilized mucilage in 1 mL of water were used for the assay. Exactly 100 µL of mucilage solution, 950 µL of distilled water, and 50 µL of Folin-Ciocalteau reagent were mixed by vortexing. After 5 min, 800 µL of Na_2CO_3 (7.5% w/v) were added and vortexed. The mixture was incubated for 1 h in the darkness at 25 ºC. The absorbance of the solution was read at 765 nm in a UV-Vis spectrophotometer (HACH DR 5000, Mexico). UV-Vis spectrophotometer (HACH DR 5000, Mexico). The phenolic content was calculated using a standard curve using gallic acid as a standard (R^2 = 0.99). The results were expressed as mg/g GAE of dry matter (dm). The analyses were performed in triplicate (González-Centeno et al., 2012).

4.4.5 Identification of polyphenolic compounds by HPLC/ESI/MS analysis

Reversed-phase high-performance liquid chromatography analyses were performed on a Varian HPLC system, which includes an auto sampler (ProStar 410, Varian, Palo Alto, CA, USA), a ternary pump (ProStar 230I, Varian, Palo Alto, California, USA) and a PDA detector (ProStar 330, Varian, Atlanta, GA, USA). An ion trap mass spectrometer for chromatography (Varian 500-MS IT Mass Spectrometer, Palo Alto, CA, USA) equipped with an electrospray ion source was also used. Approximately 10 mg of lyophilized mucilage was mixed in 1 mL of water and the solutions were subsequently filtered through a 0.45 µm PTFE filter. The samples (5 µL) were injected into a Denali C18 column (150 mm × 2.1 mm, 3 µm, Grace, Albany, OR, USA) and the oven temperature was maintained at 30 °C. The eluents were formic acid (0.2%, v/v; solvent A) and acetonitrile (solvent B). The following gradient was applied: initial, 3% B; 0-5 min, 9% linear B; 5-15 min, 16% linear B; 15-45 min, 50% linear B. The column was then washed and reconditioned; the flow rate was maintained at 0.2 mL/min and phenolic compounds were analyzed at four different wavelengths: 245, 280, 320, and 550 nm. All effluent (0.2 mL/min) was injected into the mass spectrometer source without splitting. All MS tests were carried out in the negative [M-H]-nitrogen mode was used as the nebulizer gas and helium as the buffer gas. The ion source parameters were spray voltage 5.0 kV and capillary voltage and temperature were 90.0 V and 350 °C respectively. Data were collected and processed using MS Workstation software (V 6.9). Samples were first analyzed in full scan mode acquired in the m/z 50-2000 range. MS/MS analyses were performed on a series of selected precursor ions. Finally, the compounds were compared using a bioactive compound database (WorkStation database version 2.0, VARIAN, Palo Alto, CA, USA) (Hernández-Hernández et al., 2020).

4.4.6 Antioxidant capacity

The antioxidant potential of the cactus pear mucilage samples was evaluated.
To determine the antioxidant capacity by the 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay, the methodology described by González-Centeno et al. (2012) with some modifications. ABTS (7 mmol/L) was mixed with potassium persulfate (2.45 mmol/L) 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 the antioxidant capacity, 50 µL of the mucilage extract (10 mg mucilage in 2 mL of 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). Stock solutions of Trolox were prepared at a concentration of 0.2 to 1.2 mM, using water as solvent. The results were expressed in µM Trolox equivalent/g dry matter.

To determine the antioxidant capacity by the Ferric Reducing Antioxidant Power (FRAP) method, 130 µL of the extract (10 mg mucilage in 1 mL of distilled water) were mixed with 290 µL of 0.2 M phosphate buffer (pH 6.6) and 290 µL of potassium ferricyanide at 1% (w/v). Then, the samples were incubated at 50 °C for 30 min. After incubation, 290 µL of 10% (w/v) TCA, 1 mL of distilled water, and

250 µL of 0.1% (w/v) ferric chloride were added. After 30 min of incubation at 25 °C, the absorbance at 700 nm was measured in a UV-Vis spectrophotometer (HACH DR 5000, Mexico). The Trolox calibration curve was obtained using concentrations of 0.2 to 1.2 mM. The antioxidant capacity was expressed in µM equivalent in trolox/g dry matter (Bayar et al., 2016).

4.4.7 Technological use of mucilage

Extracts and polymers obtained from plants have been used to make edible films. The mucilage of the 'Cristalina' variety under non-irrigation had the highest antioxidant capacity and total polyphenols, and therefore, it was chosen to prepare the edible coating.

4.4.8 Edible film preparation

The edible film 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 min with an Ultraturrax T18 (IKA® Works, Inc., Wilmington, USA). Subsequently, the sodium alginate emulsion was spread on a smooth glass plate and immersed in a 2% (w/v) calcium chloride solution for 3 min until a gel was formed. Finally, the film was allowed to dry at room temperature (25 °C) for 12 h. The film thickness was 0.088 ± 0.005 mm and this was measured with a Labomed VF10X optical micrometer (Labomed Inc., CA, USA).

4.4.9 Characterization of the edible film

Opacity

The opacity of the films was determined according to Kanatt & Makwana (2020) with some modifications. Rectangular films (1 x 4 cm) were cut and measured at 600 nm with a UV-Vis spectrophotometer (HACH DR 5000, Mexico). An empty cell was the reference and three replicated readings were performed. The opacity was calculated using the following formula:

$$Opacity = \frac{600\ nm}{t} \tag{8}$$

where *t* is the film thickness in mm.

Water vapor permeability (WVP)

For the determination of the WVP, the film was placed in 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:

$$WVP = \frac{WVRT}{[S(R_1 - R_2)]} D \tag{9}$$

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:

$$WVTR = \frac{\frac{dm}{dt}}{A} \tag{10}$$

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).

Mechanical properties

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 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 were analyzed with the Exponent software version 5.1.1.0 (De Moura et al., 2009).

4.4.10 Edible film application

Avocados (Hass variety) were obtained from a local supermarket (Gómez Palacio City, Durango State, Mexico). Fruits were selected free of physical damage and maturity grade 3 (fruit turned black, 50%); according to Herrera-González et al. (2017). The avocado fruits were washed with a 0.02% sodium hypochlorite solution and then manually cut into halves lengthwise. Avocado halves were randomly distributed into two batches. The first lot was coated with the alginate-mucilage film, the second lot was not coated and was taken as a control group. The avocado pieces that were coated were first impregnated with citric acid and ascorbic acid (70:30) approximately 0.1% (w/w). Subsequently, they were immersed in the film-forming emulsion for 30 s, and the excess cover was manually removed. Immediately after, the coated samples were immersed in 2% $CaCl_2$ for 1 min to gel. The control samples were immersed in distilled water. The avocado halves were placed in a current of dry air (9-10 m/s and 20 °C) for 40-50 min, to dry the formed film. The experimental samples were placed in plastic containers and stored at 4 °C and 95% RH for 12 days.

4.4.11 Effect of edible film on minimally processed avocado

Color

A Minolta CR-300 colorimeter, equipped with an included ocular component, C, and 0° viewing angle geometry, was used to obtain L* (lightness), a* (redness), and b* (yellowness). The color was measured in the pulp of 6 pieces of avocado, three times in each one of the pieces.

The total color difference (ΔE*) was calculated using the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (11)$$

Weight loss

The monitoring of the weight of the fruits was carried out in an analytical balance Ohaus, Explorer Pro (NY, USA). The weight loss value was determined by the difference between the samples at the storage times described. Approximately 300 g of avocado pieces were selected per treatment. Weight loss was reported as the percentage change from initial weight.

Firmness

This was measured by the puncture method using the Texture Analyzer TA.XT Plus texturometer, equipped with the Texture Expert Exceed 2.1 software (Microsystem, London, England); for this purpose, the 2 mm diameter punch was used at a head speed of 1.5 mm s^{-1} and a travel distance of 8 mm (Meza Velázquez et al., 2013). Punctures were made in the pulp of six pieces of avocado from each experimental unit, making four readings for each piece of avocado. Flesh firmness was defined as the maximum force to penetrate the fruit in Newtons (N).

Sensory analysis

Sensory analysis of coated and uncoated avocado pieces (control) was carried out following the methodology described by Reyes-Avalos et al. (2019). The level of liking was evaluated for appearance, color, odor, firmness, flavor, and acceptability in general in 3 samples: half fresh avocado (0 days), half control avocado, and covered with mucilage-alginate with 6 days of storage at 4 °C. Samples were labeled with 3-digit number codes that were randomly provided to the panelists. The evaluation was carried out with 30 untrained judges and regular avocado consumers. Panelists were provided with a glass of drinking water to eliminate residual taste between samples. Each attribute was scored with a

Hedonic scale labeled from "dislike very much" to "like very much".

4.4.12 Data analysis

Data were analyzed in a randomized complete block model with factorial arrangement in the treatments. Likewise, the post hoc analysis was performed using Fisher's least significant difference test at $p \leq 0.05$. While the Friedman test was used for sensory analysis. All calculations were performed in the STATISTICA® 7.0 system (StatSoft, Inc., Tulsa, OK, USA).

4.5 Results and discussion

4.5.1 Functional properties of mucilage

Functional properties of polysaccharides, such as SwI, WRC, and OHC have been related to certain health benefits, such as auxiliaries to lower blood cholesterol and glucose levels (Elleuch et al., 2011). Table 7 shows the values of SwI, WRC, OHC, and solubility of mucilage extracted from 4 varieties of cactus subjected to different irrigation regimes. It is observed that the mucilage of the varieties 'Roja Lisa' and 'Cristalina' SR, presented the highest swelling values (~6.2%) ($p \leq 0.05$); while most of the varieties, with full irrigation, had the lowest % swelling. Likewise, the varieties 'Amarilla Olorosa' and 'Dalia Roja' had the same swelling values with RS and SR ($p \leq 0.05$). On the other hand, the mucilages of the non-irrigated varieties showed higher WRC (~5.11 g H_2O/g mucilage), highlighting among them the variety 'Roja lisa' (~5.4 g H_2O/g mucilage), followed by the varieties subjected to RS; while the mucilage of the varieties irrigated with more water presented the lowest WRC (~3.8 g H_2O/g mucilage); the variety with the lowest WRC was 'Cristalina' under the RC regime (~3 g H_2O/g mucilage). The WRC and the swelling index are properties of the fibers that are related to the chemical structure of the polysaccharides that compose it and to factors such as porosity, particle size, ionic form, pH, temperature, ionic strength, and type of ions, among others. (Avlani et al., 2019; Elleuch et al., 2011; Tosif et al., 2021). The higher values of WRC and SwI are possibly due to higher molecular weight and higher contents of carboxylic acids, proteins, and uronic acids found in mucilages subjected to water stress (Luna-Zapién et al., 2023), which favors better hydration of the molecule. Interestingly, in our study, the WRC and the SwI presented a good positive correlation ($R^2 = 0.985$).

Table 7**.** Mucilage swelling index (SwI), water retention capacity (WRC), oil-holding capacity (OHC), and solubility (S) of cactus pear varieties under no irrigation (NI, control), supplemental irrigation (SI), and full irrigation (FI).

Irrigation regime	**Variety**	**SwI (%)**	**WRC (g H2O/g mucilage)**	**OHC (g oil/g mucilage)**	**S (%)**
NI	'Roja Lisa'	6.0 ± 0.5 a	5.4 ± 0.6 a	7.3 ± 0.3 f	69.1 ± 2.4 a
	'Cristalina'	6.5 ± 0.8 a	4.4 ± 0.5 bcd	7.6 ± 0.3 ef	65.4 ± 1.1 b
	'Amarilla Olorosa'	4.5 ± 0.5 b	4.93 ± 0.3 ab	7.7 ± 0.2 def	65.8 ± 0.7 b
	'Dalia Roja'	3.5 ± 0.5 bcd	4.4 ± 0.5 bcde	6.6 ± 0.4 g	63.4 ± 1.9 bcd
SI	'Roja Lisa'	3.9 ± 0.9 bc	4.8 ± 0.5 b	8.0 ± 0.3 bcde	65.6 ± 2.0 b
	'Cristalina'	3.5 ± 0.5 bcd	3.9 ± 0.4 ef	7.9 ± 0.7 cde	64.5 ± 1.1 bc
	'Amarilla Olorosa'	3.5 ± 0.53 bcd	4.2 ± 0.3 cdef	8.3 ± 0.2 bc	64.1 ± 2.6 bcd
	'Dalia Roja'	2.9 ± 0.9 cde	3.9 ± 0.3 ef	8.5 ± 0.2 b	62.1 ± 1.4 cd
FI	'Roja Lisa'	2.6 ± 0.5 de	4.6 ± 0.6 bc	8.0 ± 0.2 bcde	58.2 ± 0.8 e
	'Cristalina'	2.9 ± 0.0 cde	3.1 ± 0.3 g	8.1 ± 0.2 bcd	62.9 ± 1.8 bcd
	'Amarilla Olorosa'	3.8 ± 0.0 bc	3.9 ± 0.4 def	9.4 ± 0.8 a	63.8 ± 1.9 bcd
	'Dalia Roja'	2.0 ± 0.0 e	3.6 ± 0.3 fg	9.2 ± 0.3 a	61.1 ± 1.7 de

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

In Table 7, it can be seen that the solubility of the mucilage of the different varieties of nopal was affected by the amount of water added to the crops. Interestingly, the mucilages of the 'Roja Lisa' variety presented the highest and lowest values, being in SR and RC, respectively (69.1% and 58.2%). Also, it is shown that the solubility of the mucilages of the varieties 'Cristalina', 'Amarilla Olorosa', and 'Dalia Roja', subjected to supplementary irrigation regimes and SR, was the same. 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); the greater presence of carboxyl groups and uronic acids in the mucilage molecule of the 'Roja Lisa' variety subjected to SR (Luna-Zapién et al., 2023), could make these samples more soluble. Due to its shown solubilization capacity, the nopal mucilages investigated can be used to improve the technological properties of liquid food products; as well as, when consumed, help reduce the glycemic response and cholesterol (Abdul-Hamid & Luan, 2000; Olson et al., 1987; Roehrig, 1988).

Likewise, the results obtained OHC of the mucilages of the different varieties of Nopal NI, SI, and FI are shown in Table 7. It is observed that the varieties 'Amarilla Olorosa' and 'Dalia Roja', NI, presented the highest values of this factor ($p \leq 0.05$). Interestingly, the effect of the irrigation regime was very evident in the 'Dalia Roja' variety, since, in the FI regime, it generated the lowest OHC ($p \leq 0.05$). Likewise, the 4 varieties studied had the same OHC in SI. It is known that the functional properties of the cell wall molecules of plant materials depend on the composition of the polysaccharides that make it up and their three-dimensional structure (Jarvis, 2011). So, the scarcity or abundance of water influences, in some way, the formation of fibers with more or less polar or non-polar zones, which will make them more akin to hydrophilic or hydrophobic substances. Also, it is shown that mucilages with higher OHC had lower WRC ($R^2 = 0.99$). This coincides with what was reported by Alvarado-Morales et al. (2019), who found that aloe vera mucilages with high WRC presented lower OHC and vice versa. Cactus mucilages with high OHC could be used as stabilizers for high-fat products and/or emulsions.

4.5.2 Total polyphenolic content and identification of polyphenolic compounds of mucilage

Plant-extracted polysaccharides contain different phenolic compounds including flavonoids and polyphenols as bioactive compounds (Tosif et al., 2021). The total polyphenol content (TPC) in the mucilage of the four varieties subjected to different irrigation regimens ranged between 4.3 and 12.1 mg/g GAE dm (Table 8). The lowest TPC value was observed in 'Roja Lisa' variety under FI, while 'Cristalina' variety under NI exhibited the highest TPC value. It is known that the the concentration of phenolic compounds in the cladodes varied not only for genetic differences among *Opuntia* species included in this study (Alves et al., 2017; Bari et al., 2012), but also for the irrigation regimen (Boutakiout et al., 2018).

Table 8. Mucilage total polyphenol content (TPC) and antioxidant capacity (ABTS and FRAP methods) of cactus pear varieties under no irrigation (NI, control), supplemental irrigation (SI), and full irrigation (FI).

Irrigation regime	Variety	Total polyphenols (mg/g GAE dry matter)
NI	**'Roja Lisa'**	4.9 ± 0.0 h
	'Cristalina'	12.0 ± 0.3 a
	'Amarilla Olorosa'	10.7 ± 0.6 b
	'Dalia Roja'	8.1 ± 0.3 f
SI	**'Roja Lisa'**	4.5 ± 0.1 hi
	'Cristalina'	10.2 ± 0.3 c
	'Amarilla Olorosa'	9.7 ± 0.2 d
	'Dalia Roja'	6.8 ± 0.6 g
FI	**'Roja Lisa'**	4.3 ± 0.1 i
	'Cristalina'	9.5 ± 0.6 d
	'Amarilla Olorosa'	9.0 ± 0.3 e
	'Dalia Roja'	6.8 ± 0.1 g

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

The non-irrigated plants produced, on average, a mucilage powder with greater TPC ($p \leq 0.05$), this one increased by ~14 and 20 % concerning the mucilage with SI and FI plants, respectively. In addition, the mucilage of SI plants showed higher TPC values (~7%) than FI in all varieties studied, except for the variety 'Dalia Roja'. The increase of these compounds may be the result of the development of episodes of water stress in the plants subjected to this irrigation regimen. Some studies have observed similar behavior in cays and cladodes of *Opuntia* (Boutakiout et al., 2018; Camarena-Rangel et al., 2017). These types of plants likely develop a stronger biochemical mechanism to cope with periods of extreme drought and, consequently, an increase in the production of secondary metabolites such as polyphenols (Boutakiout et al., 2018). Differences in TPC in mucilage samples are important because these compounds have the potential for human health benefits (Gong et al., 2022; Limanaqi et al., 2020).

To our knowledge, the current study is the first to report on the polyphenolic compounds present in cactus mucilage powder. Mucilage solutions were analyzed by HPLC/ESI/MS. The compounds identified in the mucilage samples from the four-cactus pear varieties exposed to different irrigation regimes, were phloretin, pterostilbene, and sinensetin (Table 9).

Table 9. Polyphenolic compounds identified by HPLC/ESI/MS in mucilage of *Opuntia* spp. subjected to irrigation regimes.

Retention time (min)	**Compound**	**Mass (m/z)[M-H]−**	**Family**
3.29	Phloretin	272.8	Dihydrochalcones
13.09	Pterostilbene	254.9	Stilbenes
24.36	Sinensetin	371.1	Methoxyflavones

Phloretin and sinensetin have been shown to possess several potent biological activities, including anticancer, antioxidant, and anti-inflammatory effects (Fan et al., 2022; Tuli et al., 2022; Yang et al., 2022). Moreover, pterostilbene (a compound with a major presence in this mucilage) is an analog of resveratrol, which is argued to have an anti-inflammatory and antioxidant effect (An et al., 2022). Additionally, pterostilbene reduces body weight, liver fat, inflammatory biomarkers, blood glucose, and other physiologic features of metabolic disorders (Kim et al., 2020). The presence of these compounds suggests that mucilage can be considered a promising hydrocolloid to be used in the food industry.

4.5.3 Antioxidant capacity of mucilage

The antioxidant capacity of the mucilage samples was determined by the ABTS and FRAP methods; since it has been reported that a single assay is not enough to predict the antioxidant potential and that the results of the different determinations can help to elucidate the mechanism involved in the observed activities (Liguori et al., 2020). The antioxidant capacity (ABTS and FRAP method) of the mucilage of four varieties of *Opuntia* and subjected to irrigation regimes, fluctuated between 70.8 - 152.3 µM eq. Trolox /g dm and 12.1 - 58.3 µM eq. Trolox /g dm, respectively (Table 10). The differences observed between the two assays can be attributed to the variability in the hydrophilicity of the reaction mixtures, as well as the different abilities of the antioxidant compounds to reducing ABTS free radicals and ferric ions (Gentile et al., 2019). The mucilage of the 'Roja Lisa' plants presented the lowest values of antioxidant capacity, while the 'Cristalina' plants had the highest values in both methods. These differences could be explained by genetic differences among *Opuntia* species (Pretti et al., 2014). Likewise, the mucilage antioxidant capacity was the highest in all cladodes extracts of cactus pear varieties under NI ($p \leq 0.05$). The antioxidant capacity of the mucilage was higher ~16% (ABTS) and ~64% (FRAP) when the cactus pear plants were not irrigated compared with FI plants. These results suggest that water deficit promotes the increase of these compounds to withstand water deficit stress and promote tolerance to dehydration (Alves et al., 2017). Interestingly, the results show that there was a correlation between TPC and antioxidant capacity (ABTS, r = 0.96; p < 0.000; FRAP, r = 0.83; p < 0.000). This last behavior coincided with that documented by Herrera et al. (2021) for different cactus pear varieties. Thus, the mucilage of cactus plants grown in dry conditions is an important source of antioxidants, which can have various applications in the food industry, so it represents a greater opportunity for the use of *Opuntia* spp. for semi-arid regions.

Table 10. Mucilage antioxidant capacity (ABTS and FRAP methods both in µM eq. Trolox /g dry matter) of cactus pear varieties under no irrigation (NI, control), supplemental irrigation (SI), and full irrigation (FI).

Irrigation regime	Variety	ABTS	FRAP
NI	'Roja Lisa'	78.5 ± 0.8 g	15.7 ± 1.2 g
	'Cristalina'	152.3 ± 3.3 a	58.3 ± 1.7 a
	'Amarilla Olorosa'	138.0 ± 2.1 b	30.4 ± 0.5 c
	'Dalia Roja'	126.6 ± 1.5 d	21.5 ± 0.2 e
SI	'Roja Lisa'	71.0 ± 2.9 h	12.7 ± 0.8 h
	'Cristalina'	136.7 ± 5.2 bc	32.5 ± 2.2 b
	'Amarilla Olorosa'	126.3 ± 1.6 d	25.0 ± 0.9 d
	'Dalia Roja'	117.5 ± 1.3 e	19.8 ± 0.5 f
FI	'Roja Lisa'	70.8 ± 2.2 h	12.1 ± 0.9 h
	'Cristalina'	132.9 ± 2.1 c	24.1 ± 1.3 d
	'Amarilla Olorosa'	120.0 ± 3.4 e	21.8 ± 1.0 e
	'Dalia Roja'	104.9 ± 4.5 f	18.4 ± 0.3 f

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

4.5.4 Technological use of mucilage as a material for edible coatings

Characterization of the edible coating

Opacity

Edible coatings affect the appearance of food, therefore, the determination of their optical properties is essential to define if a coating can be applied to the surface of fruits and vegetables (Abdelhedi et al., 2018). For this study, the mucilage of 'Cristalina' cladode of NI plants was selected to elaborate the edible coating. The alginate-mucilage film had an opacity of 1.1 ± 0.1. This result is similar to that reported by Paula et al. (2015) on a sodium alginate-based film. Whereas, Chen et al. (2022) reported higher opacity results (5.7 ± 0.2) in a film of soy protein isolate and alginate. Previous studies have reported that adding oil and the concentration of calcium chloride led to an increase in the alginate film opacity (Gutiérrez-Jara et al., 2020; Hashemi-Gahruie et al., 2020). Low opacity values of the developed alginate-mucilage-based film, indicate high transparency and color absence, making it more likely to be accepted by consumers (Hadi et al., 2022).

Water vapor permeability (WVP)

The WVP is an important parameter to determine the water transfer from the food surface to the environment (Xu et al., 2020). The film developed here and applied to minimally processed avocados had WVP values of $2.2 \pm 0.0 \times 10^{-12}$ g m^{-1} h^{-1} Pa^{-1}. This result is lower than those documented by Abdel-Aziz & Salama (2021) in an edible film based on sodium alginate and in a film composed of sodium alginate added with *Meyerozyma caribbica* (Iñiguez-Moreno et al., 2021). The differences in WVP values can be attributed to the film preparation conditions, type of polymer, presence of hydrophobic (lipid) components, and temperature and relative humidity conditions when the WVP readings were done (Cheng et al., 2021; Shahrampour et al., 2020). Therefore, the developed alginate-mucilage film may inhibit water transfer between the food surface and the atmosphere surroundings the product, and so extending the shelf life of coated products (Fan et al., 2021).

Mechanical properties

The mechanical properties of edible films indicate the ability of the film to resist the preparation, handling, and storage of food, helping to preserve its integrity of these (Marangoni-Júnior et al., 2022). The elongation percentage of the alginate-mucilage film was 12.3 ± 3.0 %, the tensile strength had a value of 0.01 ± 0.0 MPa and Young's modulus was 0.02 ± 0.0 MPa. The findings of this study differ from those reported by Marangoni-Júnior et al. (2022) who documented a higher percentage of elongation (19.5), tensile strength (18.2 MPa), and Young's Modulus (424.0 MPa) in films based on sodium alginate and hydrolyzed collagen. However, the alginate-mucilage film presented higher Young's Modulus values compared with those found in gelatin-sodium alginate films by Liu et al. (2006). The difference in the mechanical properties of the composite films could be associated with the interaction between the molecules, which is influenced by the constituents and processing methods (Bhatia et al., 2022). On the other hand, the incorporation of lipids in the films can hinder the intermolecular interaction between polymer chains, resulting in less tensile strength materials (Mahcene et al., 2020). In addition, it has been documented that the presence of plasticizers in the film formulation weakens the intermolecular forces between adjacent macromolecule chains, reducing mechanical resistance (Liu et al., 2016).

4.5.5 Effect of alginate-mucilage coating on minimally processed avocado

Color

Color is one of the most important factors affecting consumer perception of fruits (Liu et al., 2018). The L* values decreased as the storage time elapsed in both treatments (Table 11). However, the L* parameter decreased by ~ 47% in the uncoated samples, while in the coated samples it decreased by ~ 11.3% only ($p \leq 0.05$). The decrease of the parameter L* indicates that the avocado pulp darkened (Garcia et al., 2022). Furthermore, regardless of coating application on avocado halves, a* values increased and b* values decreased, revealing that the pulp of the fruits was becoming less green and less yellow, respectively ($p \leq 0.05$) (Table 11). However, the changes in the a* parameter occurred at a much slower rate in the alginate-mucilage-coated avocado halves and did not reach the a* value of day 3 of the uncoated fruits even on the final day of storage ($p \leq 0.05$). On the other hand, from day 3 to day 12 of storage, the b* parameter in the control samples was lower than coated samples ($p \leq 0.05$). The decrease was ~ 32.5% compared with coated samples at the last day of refrigerated storage. Likewise, ΔE increased with respect to storage time in coated and uncoated avocado pulp. However, in the coated avocado halves, ΔE increased on the third day in storage. This trend remained without significant change up to the twelfth day in storage. In contrast, ΔE increased exponentially in the control samples during experimental period.

The coated avocado pieces presented a lower ΔE (~9.5) compared with the uncoated samples (~43.0) on the twelfth day in cold room storage ($p \leq 0.05$). The last finding is in agreement with that reported by Maftoonazad & Ramaswamy (2005). These authors documented that the avocado pulp coated with carboxymethylcellulose was brighter, the a* values increased and the b* values decreased to a lesser extent compared with control fruits. Thus, the color behavior in avocado pulp suggests that the alginate-mucilage coating preserves the color of the minimally processed avocado.

Table 11. Changes in color parameter values of L*, a*, b*, and ΔE* of avocado halves coated with alginate-mucilage and uncoated (control) and stored at 7 °C for 12 days.

Treatment	Time (days)	Color parameters			
		L*	**a***	**b***	**ΔE**
	0	70.9 ± 1.5 a	-13.0 ± 0.8 g	33.8 ± 0.9 a	0 ± 0.0 f
	3	47.3 ± 5.3 c	1.4 ± 1.8 c	24.8 ± 0.4 c	25.7 ± 0.1 c
Control	**6**	44.0 ± 4.5 cd	4.4 ± 0.7 b	22.7 ± 0.9 d	31.4 ± 1.1 b
	9	40.0 ± 5.2 de	5.3 ± 1.3 ab	21.5 ± 2.0 d	34.8 ± 2.5 b
	12	37.7 ± 4.1 e	6.3 ± 0.9 a	18.4 ± 0.3 e	42.9 ± 1.9 a
	0	69.0 ± 1.6 a	-8.3 ± 0.6 f	34.1 ± 0.8 a	0 ± 0.0 f
	3	67.1 ± 0.9 ab	-6.2 ± 0.7 e	31.2 ± 1.2 b	4.2 ± 0.4 ef
Coated	**6**	65.7 ± 0.8 ab	-4.4 ± 0.4 d	31.2 ± 0.7 b	6.0 ± 0.6 de
	9	63.1 ± 2.6 b	-3.8 ± 0.2 d	31.0 ± 1.6 b	8.1 ± 0.4 de
	12	62.1 ± 1.8 b	-3.5 ± 0.2 d	29.7 ± 1.1 b	9.5 ± 0.3 de

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

Therefore, this coating had a positive effect on the visual fruit quality (Figure 6). The reduced color changes in the coated fruit may be associated with the effect of the coating in creating a protective barrier and a modified atmosphere within the fruit (Maftoonazad & Ramaswamy, 2005). This barrier reduces gaseous oxygen exchange and reduces oxidative stress and peroxide anion (O_2^-) and hydrogen peroxide (H_2O_2) levels, which are necessary for darkening development (Wang et al., 2021a).

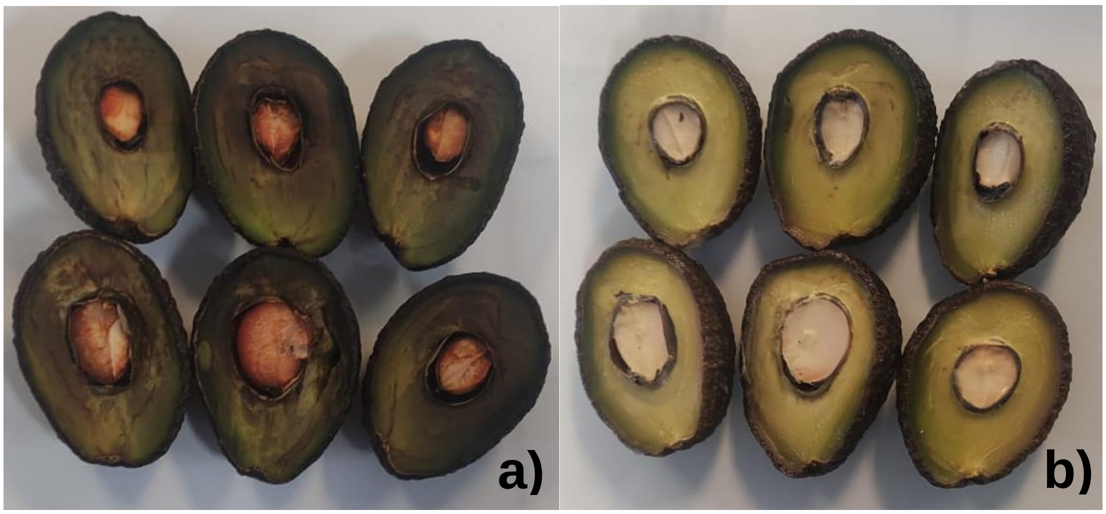

Figure 6. Avocado halves were stored for 9 days at 7°C control (uncoated) (a) and coated with alginate-mucilage (b).

Weight loss

Moisture loss causes shrinkage and weight loss in fruits and vegetables, mainly affecting minimally processed products, thus affecting their quality negatively (Rossi-Marquez et al., 2017). For this reason, weight loss was evaluated in avocado fruits with and without coating application. The main mechanism of moisture loss from fresh fruits and vegetables is vapor phase diffusion driven by a water vapor pressure gradient between the inside and outside of the fruit (Maftoonazad & Ramaswamy, 2005). However, lower weight loss values were observed in coated avocado halves compared with uncoated avocado halves from the third day in storage ($p \leq 0.05$). By the twelfth in storage, the coated avocado samples lost ~ 5 % less weight than the control samples (Figure 7). A similar trend has been observed in other minimally processed fruits such as papaya cubes (Tabassum & Khan, 2020) and apple slices (Chen et al., 2021). Thus, the alginate-mucilage coating controls fruit weight loss and vegetables, probably by acting as a semi-permeable barrier against oxygen, carbon dioxide, and moisture loss, thus reducing respiration and oxidation reactions (Cazón et al., 2017).

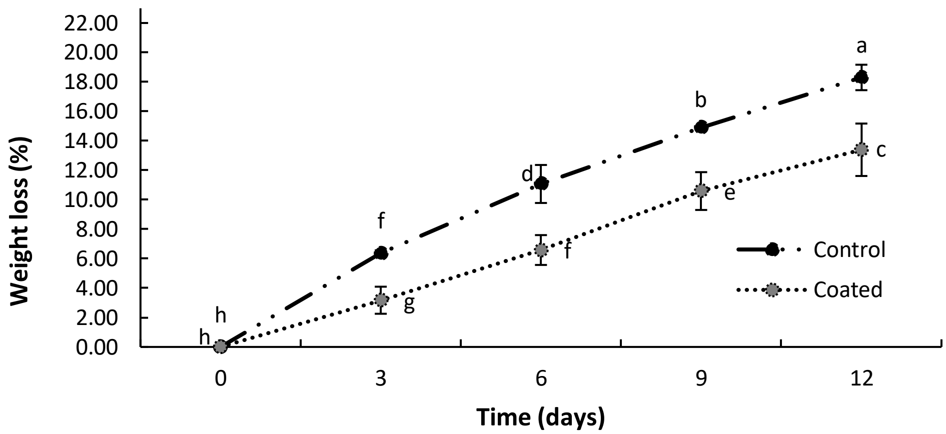

Figure 7**.** Changes in weight loss (%) of coated and uncoated avocado halves stored at 7 °C for 12 days. Mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to the Fisher's least significant difference (LSD) test at $p \leq 0.05$.

Firmness

Figure 8 shows the changes in the firmness of the avocado halves as a function of the storage period. The firmness of coated and uncoated avocado samples increased during storage time ($p \leq 0.05$). However, the firmness of the coated avocado samples was maintained until the sixth day, but a significant increase was observed on the ninth day. In the uncoated fruit, firmness gradually increased during the time of the study. On the twelfth day in storage, the coated samples had greater firmness values (3.03 N) compared with the control samples (7.8 N). The increase in firmness in minimally processed fruit has been previously documented by Basaglia et al. (2021). These authors observed a significant firmness increase in control pineapple cubes over the days of storage compared with pineapple cubes coated with chitosan and cinnamon essential oil. In this study, increased firmness in uncoated avocado pieces could be associated with the formation of tough surface tissue due to increased moisture loss, causing a hardening of the minimally processed fruits during storage (Duan et al., 2011; Souza et al., 2005).

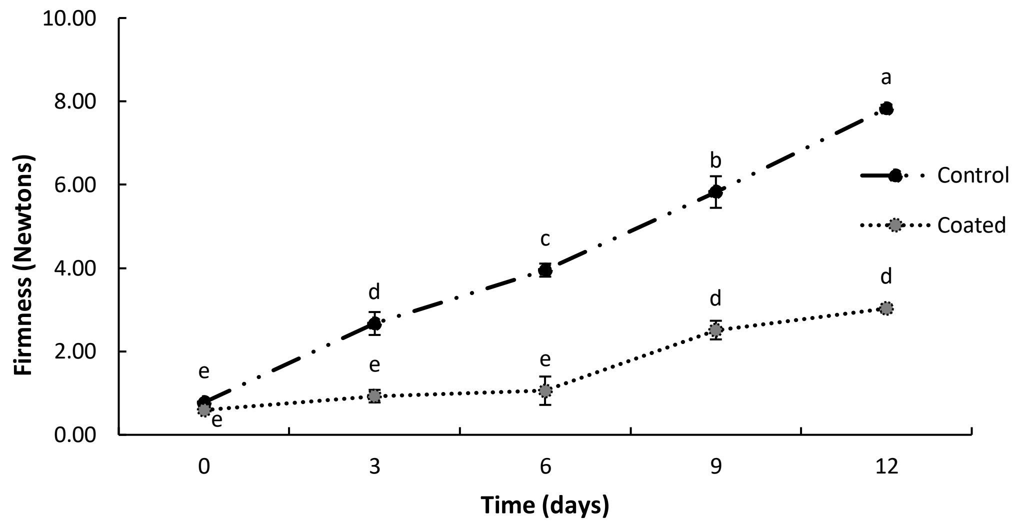

Figure 8. Changes in firmness (Newtons) of uncoated and coated avocado halves stored at 7 °C for 12 days. Mean values (± standard deviation; $n = 3$) with different letters indicate statistical differences according to the Fisher's least significant difference (LSD) test at $p \leq 0.05$.

Sensory analysis

Sensory analysis is important when studying the feasibility of adding functional ingredients to edible coating formulations that will be applied to fruits and vegetables (Alvarez et al., 2021). The results of the sensory analysis of minimally processed avocado coated and uncoated with alginate-mucilage are presented in Figure 9. In general, fresh-cut avocado samples exhibited higher acceptance in all attributes evaluated compared with coated and uncoated avocado halves ($p \leq 0.05$). For appearance and firmness, coated avocado samples reported higher sensory scores (5.4 and 6.1) than uncoated samples (1.8 and 3.6). Concerning color, the coated avocado pieces showed three times higher scores compared with the control samples.

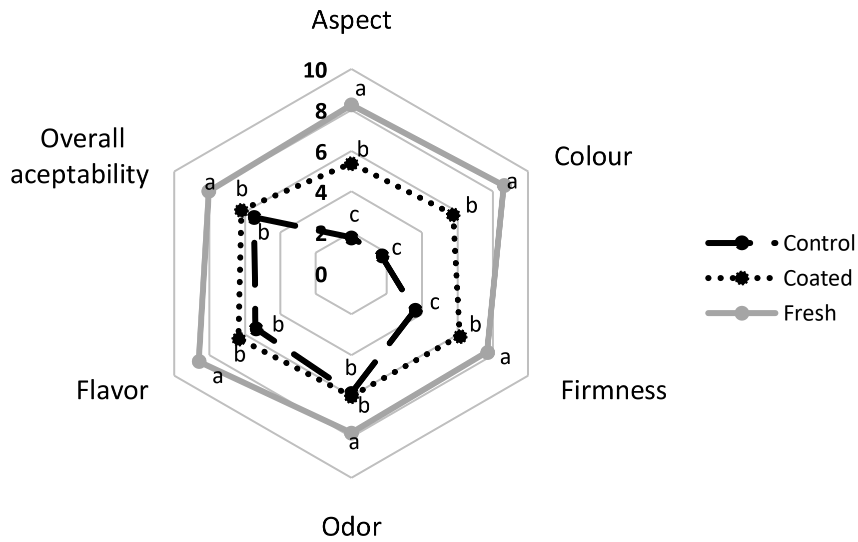

Figure 9. Sensory attribute ratings of coated and uncoated avocado stored at 7 °C for 6 days. Differences between means (*n*= 30) were established by Fisher's test ($p \leq 0.05$). Values followed by different lowercase letters are statistically different.

The great acceptance of the judges was probably due to the beneficial effect of the coating on the color attribute preservation in the avocado halves' surfaces. The sensory attributes, such as smell, taste, and acceptability in general presented similar results in the coated and control avocado halves ($p \leq 0.05$). In such a way, the alginate-mucilage coating did not contribute foreign flavors or odors to the avocado fruits. Similar behavior has been observed in pineapple cubes coated with sodium alginate and citral nanoemulsión (Prakash et al., 2020) and in papaya cubes with an alginate-based coating added with quercetin glycoside and hydroxyapatite (Montone et al., 2022). Therefore, the avocado pieces with alginate-mucilage coating reached values suitable for commercial purposes by the sixth day.

4.6 Conclusions

Irrigation and *Opuntia* varieties significantly influenced the functional properties and bioactive compounds of the mucilage. In general, the functional properties, the content of phenolic compounds, and the antioxidant capacity of the mucilage of NI plants were higher compared with SI and FI plants. The mucilage powder of the 'Cristalina' plants under NI produced the highest swelling index value, while ‘Roja Lisa' and 'Amarilla Olorosa' plants under NI had the highest values of water retention curve and solubility. On the contrary, the mucilage of the 'Dalia Roja' plants under FI had the highest oil holding capacity value. Likewise, the mucilage of the 'Cristalina' plants under NI exhibited the highest total polyphenols and antioxidant capacity values (ABTS and FRAP methods). Therefore, restricting the amount of water to the cactus varieties studied here could allow the construction of strategies to select drought-tolerant varieties; in addition to being a valuable perspective for regions with limited agricultural opportunities. In addition, the characteristics of the mucilage observed in the present study suggest a potential use for the food industry.

The alginate-mucilage-based coating applied to avocado halves reduced weight loss, color, and firmness changes. In addition, the sensory analysis showed that coated avocado halves exhibited greater acceptance of the color and firmness attributes. Therefore, the edible alginate-mucilage coating could be considered a potential and effective alternative to preserve the commercial quality of avocado halves. Likewise, the alginate-mucilage coating can be a feasible option to extend the shelf life of other fresh-cut fruits and vegetables with global economic importance.

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5. GENERAL CONCLUSIONS

Fourier Transform Infrared Spectroscopy study of all mucilages shows characteristic signals of a polysaccharide and the resulting mucilage structure probably corresponds to a xyloglucan skeleton of type XXGG (Xyl, Xyl, Glc, Glc, Glc) with arabinose branching.

Statistical evidence confirmed the hypothesis that irrigation modifies some physical, chemical, and functional characteristics of mucilage extracted from cladodes of different cactus pear cactus varieties. The mucilage yield of non-irrigated plants exhibited the highest values. Likewise, the color of the mucilage powder from plants under the same irrigation conditions had the highest saturation value (L*) with lower values of red to green (a*) and blue to yellow (b*) in darkness, higher protein, fiber, total carbohydrate, glucose, arabinose, and uronic acids, but lower ash content. In addition, viscosity and molar mass increased proportionally in plants receiving no watering.

On the other hand, the 'Amarilla Olorosa' and 'Roja Lisa' varieties grown without irrigation produced the highest mucilage yield. The mucilage from 'Cristalina' and 'Dalia Roja' plants exhibited the highest protein and ash content, but the lowest carbohydrate and total fiber values compared with ‘Amarilla Olorosa’ and ‘Roja Lisa’ varieties. The mucilage solutions from 'Amarilla Olorosa' plants with no irrigation had the highest molar mass and viscosity. In addition, the oil holding capacity was higher in the mucilage with fully irrigated plants of 'Amarilla Olorosa' and 'Dalia Roja'. The mucilage of 'Roja Lisa' plants without irrigation produced high values of solubility and water-holding capacity, while the mucilage powders of 'Cristalina' plants without irrigation produced the highest swelling index, total polyphenol content, and antioxidant capacity.

In addition, the alginate edible film in combination with ‘Cristalina’ mucilage produced under no irrigation conditions and applied to avocado fresh-cut halves reduced weight loss and minimized changes in both color and flesh firmness. Therefore, positively increased the acceptance of this treated product by consumers.

The results suggest that restricting the amount of water either applying supplemental irrigation or no irrigation to cactus pear plants can be a feasible strategy to obtain good quality mucilage for different agro-industry purposes in arid and semi-arid zones. The characteristics observed in all mucilages extracted and purified have great potential as food additives or functional properties that can be used in the edible film formulations to extend the shelf life of fruits and vegetables minimally processed like in avocado halves studied here.