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Physicochemical and rheological properties of mucilage extracted from *Opuntia ficus indica* (L. Miller). Comparative study with guar gum and xanthan gum

Claudia Quinzio[1,2] · **Carolina Ayunta**[1,2] · **Matías Alancay**[1,2] · **Beatriz López de Mishima**[1,3] · **Laura Iturriaga**[1,2]

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Abstract The physicochemical and rheological properties of aqueous solutions of the mucilage isolated from *Opuntia ficus indica* (L. Mill) at different concentration (0.5, 1, 1.5 and 4.5% w/v) were examined. The intrinsic viscosity [η] found for precipitate mucilage (PM) and dialyzed mucilage (DM) were 22.6 and 15.3 dl/g respectively. Electrophoretic measurements showed that the zeta potential of PM and DM was negative in all the pH range studied. PM reduced the surface tension of water and was concentration dependent. The surface activity of PM (57 mN/m) was similar to that of guar gum (55 mN/m) and xanthan gum (52 mN/m) at 1.5% (w/v) concentration. A non-Newtonian shear-thinning behavior was observed. The Ostwald–de Waele model successfully correlated the viscosity–shear rate. At equal hydrocolloid concentration, the consistency coefficients (*k*) of mucilage solutions were lower than those of guar gum (GG) and xanthan gum (XG). However, the consistency coefficient of a mucilage solution at 4.5% (w/v) (20.9 Pa s) was in the same order as that shown by GG at 1.5% (w/v) (28.8 Pa s) aqueous solution. No effects of pH and ionic strength on the viscosity of PM and DM were observed. The mechanical spectra showed a crossover point between G′ and G″ at low mucilage concentrations of 0.5 and 1% (w/v), exhibiting higher relaxation time than GG. The more concentrated solutions of PM and DM (1.5 and 4.5% w/v) displayed a predominant elastic behavior and did not meet the Cox-Merz rule, similarly to GX. The more concentrated solutions of PM and DM did not meet the Cox-Merz rule. This behavior would suggest the formation of colloidal aggregates.

Keywords Mucilage · Physicochemical characterization · Rheological properties · pH · Ionic strength · Viscosity

Introduction

Opuntia ficus indica (L. Miller) is a tropical or subtropical plant, which belongs to the Cactaceae family. In Argentina, the *O. ficus indica* (L. Mill) farming is widespread mainly in the Norwest region of this country, traditionally in small plantations although the tech cultivation has increased [1, 2].

The plant is mainly used for fruit production, but in some countries, it is also used as a vegetable (nopalitos) for human consumption or as fodder. It can be used in more integral form, taking advantage of the interesting properties of the vegetative part of the plant (cladodes). The Cactaceae family is characterized by its mucilage production [3].

The first suggested structure describes the molecule as a branched polysaccharide β-(1→4)-D-galacturonic acid and α-(1→2)-L-rhamnose as core chain. The side chains are formed by the skeleton of β-(1→6)-D-galactose, which is attached to the rhamnose backbone. The galactose residues have side branches with additional units of arabinose, xylose and galactose [4]. The presence of D-galacturonic acid has been a cause of confusion for several authors who have referred to the mucilage as pectin or a pectinoid [5, 6]. In contrast, Amin et al. [7] found that the mucilage

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✉ Laura Iturriaga
laura.iturriaga@gmail.com

[1] Research and Transfer Center of Santiago del Estero (CITSE, UNSE-CONICET) RN 9 Km 1125 Villa el Zanjón, Santiago del Estero, Argentina

[2] Institute of Food Science and Technology, National University of Santiago del Estero, Av Belgrano (S) 1912, Santiago del Estero, Argentina

[3] Institute of Bionanotechnology (INBIONATEC, UNSE-CONICET), National University of Santiago de Estero, RN 9 km 1125 Villa el Zanjón, Santiago del Estero G4206XCP, Argentina

is a neutral polysaccharide of approximately 55 sugar residues without uronic acids. The mucilage consists of a complex mixture of glycoproteins and heteropolysaccharides [8], which have been fractionated, but characterized only in part. According to these authors the differences found in the results could be due to contaminations of the mucilage with other compounds originating in the cellular wall, and/or other purification methods that were not completely effective. To our knowledge, a complete characterization of the molecular structure of mucilage has not been reported.

Polysaccharide extracts from plants, like those from the Cactaceae family, represent an important source of additives for several industries, especially in the food and drug industry. Trombetta et al. [9]; Chaoucha et al. [10] studied different fractions of polysaccharides extracted from *O. ficus indica* (OFI) and found that these polysaccharides may have potential biological activity such as antioxidant and antiglycated activity, and the extraction under acidic conditions may enhance these properties.

In recent decades, the use of polysaccharides in food has increased significantly, which has stimulated the search for new hydrocolloids with novel functional properties such as encapsulating agent or economic advantages over existing ones [11]. Hydrocolloids, whose viscosity is not greatly influenced by changes in pH, the presence of salts or temperature when they are added to complex food systems, are particularly important in the food industry.

Some scientific investigations on the extraction and analysis of mucilage [4, 5, 12] and its biophysical properties [13] have reported that the mucilage is a polyelectrolyte with good viscous properties depending on the concentration, pH and ionic strength. However, the behavior of mucilage, particularly compared to that of commercial food hydrocolloids, has not been investigated enough.

The aim of the present study is to characterize the physicochemical and rheological properties of mucilage gum extracted from *O. ficus indica* (L. Mill) and compare it with hydrocolloids commonly used in the food industry, such as guar gum and xanthan gum. The main topics to be examined here will be the effects of concentration, pH and ionic strength on the flow and viscoelastic properties.

Materials and methods

Materials

Two-year old cladodes of *O. ficus indica* (L. Mill), from the El Zanjon cultivar belonging to the School of Agronomy and Agribusiness of the National University of Santiago del Estero, Argentina, were used. Food grade guar gum (GG) and xanthan gum (XG) were used as well.

Mucilage extraction and purification

The natural exudate of diced parenchymatous tissue from cladodes was extracted using a stainless steel extruder and centrifuged at 10,000 rpm for 1 h. The supernatant was precipitated using five volumes of ethanol. Afterwards, the precipitate was dried in an oven at 60 °C up to constant weight (precipitated mucilage, PM). A water suspension of a PM sample was dialyzed for 24 h at 4 °C using a cellulose membrane of 12 kDa cutoff against two changes of distilled water. This purified sample was precipitated and dried, as was described for PM and is referred to as dialyzed mucilage (DM).

Centesimal composition of the mucilage

The chemical composition of PM and DM was determined using [14] techniques: moisture (C.A.A 13.21, 1989), protein content (C.A.A 928.08, 1990) and ash content (C.A.A 923, 03, 1990). The minerals (Na^+, K^+, Ca^{2+}, Mg^{2+}) were quantified using a flame photometer, model Crudo Ionometer.

Physicochemical characterization of the mucilage

Preparation of solutions

Aqueous solutions at 0.5, 1.0 and 1.5% (w/v) of PM, DM, GG and XG were prepared by dissolving the appropriate quantity of the biopolymer in distilled water. Solutions of PM and DM at 4.5% (w/v) were also prepared. The solutions were stirred using a magnetic stirrer for 6 h. at room temperature and set to rest for 24 h at 4 °C in order to dissolve the polysaccharides completely.

Solutions of PM and DM at 1.5% (w/v) were prepared at different pH values (3–11) and at different ionic strengths (0–0.6 M). The following buffers were used: $C_2H_4O_2$/$C_2H_3NaO_2$ (0.2 M/0.2 M); NaH_2PO_4/Na_2HPO_4 (0.2 M/0.2 M); H_3BO_3/KCl/NaOH (0.2 M/0.2 M/0.2 M) [15]. The desired ionic strength was adjusted using different concentrations of KCl, NaCl, $CaCl_2$ and $MgCl_2$ solutions.

Intrinsic viscosity

Mucilage solutions (PM and DM) at 0.02; 0.04; 0.06; 0.8 and 0.1 dl/g were prepared in distilled water. The viscosity of dilute solutions was measured using a Micro-Ubbelohde viscometer (DIN 55 350) immersed in a 25 °C water bath.

The intrinsic viscosity [η] was determined from the extrapolation of the reduced $\left(\eta_{red}\right)_{c\to 0}$ and inherent $\left(\eta_{inh}\right)_{c\to 0}$ viscosity at zero concentration in accordance with the empirical expressions of the Huggins and Kraemer Eqs. (1) and (2), respectively.

$$\eta_{red} = \frac{\eta_{sp}}{c} = [\eta]_H + K_H[\eta]_H^2 c \quad (1)$$

$$\eta_{inh} = \ln\frac{\eta_{rel}}{c} = [\eta]_k + K_k[\eta]^2{}_k c \quad (2)$$

Viscosimetric average molecular weight

The viscosimetric average molecular weight ($\overline{M}_v$) was determined using the Mark–Houwink Sakurada equation,

$$[\eta] = K\left[\overline{M}_v\right]^{\alpha} \quad (3)$$

where [η] is the intrinsic viscosity (dl/g), $\overline{M}_v$ is the viscosimetric average molecular weight, K and [α] are constants for a given solute–solvent system and for a given temperature.

Zeta potential

Aqueous 0.1% (w/v) PM and DM solutions were prepared and stored at 4 °C for 24 h in order to allow their complete hydration. The zeta potential of the biopolymer aqueous solutions was determined at different pH values using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK), which includes a microprocessor that first measures the electrophoretic mobility of colloidal particles dispersed in aqueous solutions, and then automatically calculates the zeta potential using the Smoluchowski equation. The pH of the solutions was adjusted by the addition of 0.1 N HCl and/or 0.1 N NaOH.

Surface tension

The surface tension (mN/m) of the air-aqueous dispersion was measured by a method described by Izydorczyk, Biliaderis, and Bushuk [16] using Kibron Micro Trough S. Solutions of different hydrocolloids at 0.5, 1, 1.5% (w/v) were placed in the measuring cuvette equipment. Changes in surface tension were detected by a probe in contact with the interface. Changes in surface tension were recorded every 5 min for 2 h at 25 °C. Reading and data analysis was performed through the Film Ware software.

Rheological behavior

All the rheological determinations were performed in an AR1000 TA Instruments rheometer (New Castle, DE197020), at 25 °C, using stainless-steel cone-plate geometry (60 mm diameter and 2°angle) running under the LLC 109 TA Instrument-Water Lukens Drive. Data were collected by running the 5.2.18 release of the Rheology Advantage Data Analysis software.

Steady-shear flow measurements

The flow behavior of all the samples at different concentrations was studied. Additionally, mucilage solutions at 1.5% (w/v) were evaluated in terms of pH and ionic strength.

Steady-state flow curves were obtained using a one-step program (0–300 s^{-1}). The experimental flow curves were modelled using the Ostwald-de Waele model, as expressed in the following equation:

$$\eta = k\dot{\gamma}^{n-1} \quad (4)$$

where k is the consistency coefficient (Pa s^n), [$\dot{\gamma}$] is the shear rate (s^{-1}) and n is the index of flow behavior (dimensionless).

Steady oscillatory flow measurements

The dynamic moduli, G′ (storage modulus) and G″ (loss modulus), were determined through small amplitude oscillatory shear flow at frequencies ranging from 0.01 to 10 Hz, and constant stress of 1 Pa, within the linear viscoelastic range (LVR).

Statistical analysis

In order to find significant differences, a one-way variance analysis (ANOVA) as well as tests of mean differences for a significance level of $\alpha = 0.05$ using the Tukey's test were carried out.

The tests were done in triplicate using the Info Stat software.

Results and discussion

Mucilage extraction and purification

The procedure for the extraction and purification of mucilage from cladodes is presented in Fig. 1. The yield reached was 0.15% (w/w) of whole cladode and about 1.5% (w/w) on dry basis, which is greater than 0.07 g/100 g fresh whole cladodes and lower than 1.3–1.6 g/100 g fresh cactus reported by Cárdenas et al. [17] and Sepúlveda et al. [18], respectively. These differences could be related to the method employed, times of extraction, solvents used for the precipitation, and the crop age [17, 19]. Besides, the yield of the process would depend on the synthesis

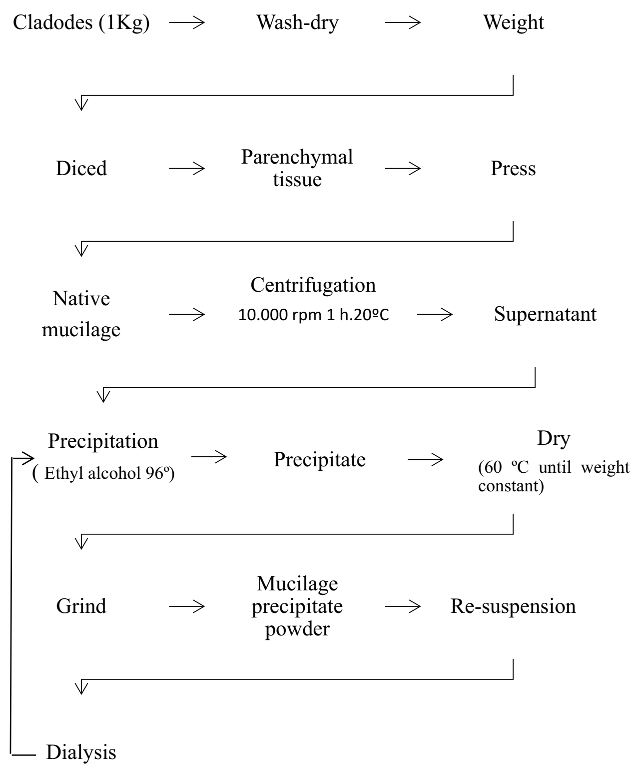

Fig. 1 Laboratory-scale protocol for the extraction and purification of mucilage from Opuntia cladodes

of mucilage that varies with climatic conditions, being higher under water stress [3]. In this sense, the highest yield obtained in this work (0.19% w/w) was recorded in July, a month when the less abundant regional rainfalls occur (Santiago del Estero, 27°45′S–64°16′W, Argentina) and the lowest one (0.13% w/w) was recorded in the rainy season (December).

Table 1 Chemical composition and mineral content of PM and DM

	PM^{n}	DM^{n}
Moisture (%)	$9.3^{a} \pm 0.1$	$11.9^{b} \pm 0.5$
Protein (%)	$2.7^{a} \pm 0.1$	$2.7^{a} \pm 0.1$
Ash (%)	$9.8^{a} \pm 0.3$	$6.4^{b} \pm 0.4$
Na^{+} (mg/100 g)	$0.40^{a} \pm 0.05$	$0.30^{b} \pm 0.02$
K^{+} (mg/100 g)	$1.3^{a} \pm 0.1$	$1.02^{b} \pm 0.02$
Ca^{2+} (mg/100 g)	$6.2^{a} \pm 0.1$	$3.2^{b} \pm 0.4$
Mg^{2+} (mg/100 g)	$0.80^{a} \pm 0.05$	$0.59^{b} \pm 0.03$

Values with same letter in a row are not significantly different for a level of $\alpha < 0.05$ significance for the average difference test of Tukey. Values are expressed on a dry basis.

[n] Average value of three replicates

Centesimal composition of the mucilage

Table 1 lists the moisture, ash, minerals and proteins contents corresponding to PM and DM. The ash content shown in Table 1 is significant since it is higher than the protein contents on a dry basis. Reports with higher and lower ash contents can be found and have been related to the various extraction processes, solvents used, the age of cladodes and the crop region, among others [17–19]. The ash content decreased in the DM samples probably due to the dialysis step. Considering that ashes represent the minerals; different cations were measured. Results indicated that Ca^{2+} was the major mineral followed by K^{+}, Mg^{2+}, and Na^{+}. Since Ca^{2+}, which has a significant effect on the water-retention capacity and other biophysical properties of Opuntia mucilage, is usually present in greater proportion [20]. The content of Ca^{2+} in DM decreased to half compared to that of PM. As regards the Mg^{2+} content, a higher value (8.4%) than that listed in Table 1 was reported by Aguilar-Chavez [21]. Mg^{2+} is associated with chlorophyll and is present in the chlorenchyma tissue of cladodes (rich in chlorophyll), so the low value of Mg^{2+} determined in the present work would be because the mucilage was obtained from parenchymal tissue. The values reported by Aguilar-Chavez [21] refer to mucilage extracted from whole cladodes.

Regarding the protein content, mucilage samples showed a value (2.7%) similar to that of guar gum (2.56–3.46%), but higher than that reported for xanthan gum (0.86%) [22, 23]. López [24] reported a protein content of 3.7% in 3-year-old cladodes and 9.4% for younger ones. Nobel [25], Flores and Brauer [26] reported values from 4.3% of protein in 8-month-old cladodes to 6.2% of protein in 3-month-old cladodes. Dickinson [27] reported that many polysaccharides, such as Arabic gum, galactomannans (fenugreek, guar, tara, locust bean gums) and pectin, contain a residual strongly bound protein fraction, which was associated with some functional properties such as surface activity and emulsification ability.

Table 2 Intrinsic viscosity (dl/g) obtained by Huggins and Kraemer models to PM and DM solutions

	PM	DM
$[\eta]^{n}$ Huggins (dl/g)	$22.6^{a1} \pm 1.0$	$15.3^{b2} \pm 0.7$
$[\eta]^{n}$ Kraemer (dl/g)	$20.4^{a1} \pm 1.3$	$14.4^{b2} \pm 0.7$
K_H	0.05 ± 0.01	0.10 ± 0.01
K_k	-0.19 ± 0.01	-0.21 ± 0.01

Values with same letter in a row and the same number in the same column are not significantly different. The test of mean differences for a significance level a = 0.05, using Tukey's test

[n] Average value of three replicates

Physicochemical characterization of the mucilage

Intrinsic viscosity

Table 2 lists the values of intrinsic viscosity for each sample that were obtained by extrapolation to infinite dilution of both Eqs. (1) and (2).

The DM showed a significantly lower intrinsic viscosity than PM, probably due to the double precipitation to which DM was subjected [28]. Trachtenberg and Mayer [13], determined a similar value of [η] for mucilage of cladodes ([η] ~ 20 dl/g in H_2O). PM and DM intrinsic viscosity values were higher than those informed for guar gum, [η] = 10.30 dl/g [29], and lower than that of xanthan gum ([η] = 155.7 dl/g) [30]. Intrinsic viscosity is a parameter reflecting the hydrodynamic volume occupied by the polymer. Higher values are associated with a rigid-stick conformation and lower values with a more flexible conformation [31]. In this sense, the values found for PM and DM would indicate the case of a flexible molecule, similar to guar gum.

The lower than 0.5 of the PM and DM Huggins constant listed in Table 2 would indicate that water is a good solvent for this polymer [32], in line with the negative values for the Kraemer constant determined in this work [33]. These values of the Huggins and Kraemer constants indicate that the mucilage molecule would show a flexible and extended conformation where the polymer–water interactions are stronger than the polymer–polymer interactions.

Viscosimetric molecular weight

Considering that the mucilage has a polydispersity index of 1.4 [13] that correspond to a polysaccharide whit low polydispersity, the viscosimetric average molecular weight ($\overline{M}_v$) of PM and DM were determined using the equation of Mark–Houwink–Sakurada.

Assumed a “random coil” configuration for mucilage aqueous solutions [34], values of α (0.723) and K (3.810^{-4} dl/g) corresponding to flexible molecules were used [35]. The $\overline{M}_v$ of PM and DM was 3.9×10^6 and 2.3×10^6 Da, respectively. These values are in the same order of magnitude as the average molecular weight ($M_w = 4.3 \times 10^6$ Da) informed previously by Trachtenberg and Mayer [5] using ultracentrifugation experiments. Studies done by Cárdenas et al. [17] indicated that the molecular weight of this hydrocolloid, assessed by SEC-HPLC, is 3×10^6 Da, which is smaller than that indicated by Trachtenberg and Mayer [5]. Medina-Torres et al. [34] informed an M_w of 2.3×10^4 for the mucilage. They attributed the differences between their M_w and those reported in the literature to the various techniques of isolation and contamination of the mucilage with other cell compounds [3]. However, using steric exclusion chromatography (SEC/MALLS), Majdoub et al. [12] determined the molecular weight of purified samples of nopal cactus pulp and nopal peels; the values found were 6.12×10^6 and 2.53×10^6, respectively.

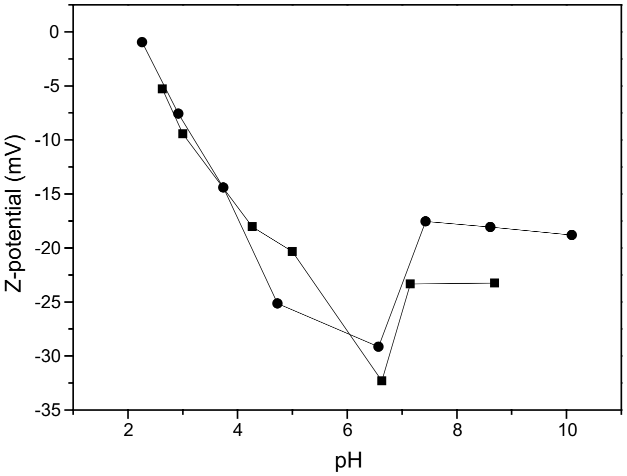

Fig. 2 Zeta potential of filled square PM and filled circle DM versus pH

Zeta potential

The measurement of electrophoretic mobility (expressed as z-potential) is a useful method to characterize the surface charge properties of protein as well as hydrocolloids [36, 37]. The z-potential of PM and DM samples was measured at pH values between 2 and 10 (Fig. 2). It was negative for both PM and DM throughout the pH range studied, which means that most of the carboxylic groups are ionized.

The negative z-potential decreased as the pH increased to a minimum of − 32 mV for PM and − 30 mV for DM at pH = 6.5 and tended to plateau at pH > 7 for both samples. After pH 6.5 DM had a less negative z-potential than PM. The difference between the z-potential values can be due to different surface charge densities for mucilage molecules with the same conformation. However, it is also possible that the zeta potentials are similar and the differences are derived from unlike conformations. The DM has a smaller hydrodynamic volume than PM, a less expanded molecule, the carboxyl groups may be less exposed, and therefore, there is less negative charge on the surface. This could be explained more by a surface phenomenon than by a difference in the amount of negative charges.

These results showed that the mucilage is an anionic polyelectrolyte where the negative charge would correspond to the carboxylic groups of galacturonic acid that forms the backbone of this polysaccharide. The zeta potential values of mucilage were in the same range as that of pectins, which also have a negative z-potential in the pH range 2–10 [38].

Surface tension

The surface tension decreased on increasing the concentration of mucilage samples in the range 0.5–1.5% (w/v). The same behavior was observed for GG and XG. At the maximum concentration studied (1.5% w/v) the surface activity was 57, 55 and 52 mN/m for PM, XG and GG, respectively. The DM surface tension decreased slightly.

It has been reported that the surface activity of polysaccharides may be caused by the presence of some hydrophobic groups such as methoxy and acetyl groups, the presence of proteins and the smaller molecular weight fractions [27, 39].

Therefore, the surface activity of PM was probably related to the presence of protein bound to the polysaccharide (Table 1). In the case of DM, the higher interfacial activity, which was in the range of 72–70 mN/m, might be a consequence of its less expanded molecule and fewer carboxylic groups exposed, as was postulated in the discussion of zeta potential.

The reliability of the published literature on the apparent surface activity of certain hydrocolloids is undermined somewhat by the fact that many commercial gum samples contain a small amount of proteins, either as contaminant or as intrinsic part of the molecular structure. As this proteinaceous material is typically strongly hidrophobic, it can adsorb strongly at liquid interfaces [27]. Ellis et al. [40] informed that the surface activity of GG can be related to protein content and to the high number of galactose branches.

Rheological behavior

Steady-shear flow measurements

Concentration effect Table 3 shows the parameters k and n obtained by linear regression analysis according to Ostwald-de Waele model for the flow curves of PM and DM solutions. The k values increased as the concentration increased and they were higher for PM than for DM at the same concentration. All the samples showed values of $n < 1$ characteristic of shear-thinning behavior, which became progressively more pronounced as the mucilage concentration increased. Cárdenas et al. [17] studied the mucilage rheology and reported shear-thinning behavior, typical of polymers that adopt a random, disordered coil conformation [41]. Medina-Torres et al. [34] reported a similar behavior, although the k values were lower and n higher, which can be explained considering the lower molecular weight of their mucilage molecule. Majdoub et al. [42] also investigated the rheological properties of cactus extracts; however, their extraction methodology was significantly different from that in preceding research, involving significant ultrafiltration of the product. They extracted two fractions; a high molecular weight extract with polysaccharides and galacturonic acid (which they thought to be pectin) and a low molecular weight extract thought to be a protein. Rheological trials on their high weight sample showed shear-thinning, which was significant in the presence of Ca^{2+} ions. The consensus was that shear-thinning was due to a random coil formation of the polysaccharide polymer components [12–34].

Table 3 Ostwald de Waele parameters and relaxation time for PM, DM and commercial hydrocolloid solutions at different concentrations

Hydrocolloid	Concentration (% w/v)	k (Pa s^{n})	n	Relaxation time[n] (s)
PM	0.50	0.30	0.52	1^{a}
	1.0	3.31	0.43	50.2^{b}
	1.50	9.78	0.36	–
	4.5	20.86	0.23	–
DM	0.50	0.22	0.54	0.6^{c}
	1.0	0.65	0.47	1.6^{d}
	1.50	1.52	0.39	2.5^{e}
	4.50	18.94	0.25	–
GG	0.50	1.85	0.47	0.2^{f}
	1.0	13.45	0.29	1.4^{d}
	1.50	29.09	0.27	4.1^{g}
XG	0.50	2.35	0.21	–
	1.0	14.49	0.21	–
	1.50	30.39	0.18	–
CMC	0.50	0.06	0.93	–
	1.0	1.80	0.80	–
	1.50	3.34	0.66	–

Values with same letter in the same column are not significantly different. The test of mean differences for a significance level a = 0.05, using Tukey's test

[n] Average value of three replicates

The results of mucilage were compared to those of the aqueous solutions of GG and XG whose parameters are included in Table 3. It should be noted that the steady-shear viscous flow behavior of these hydrocolloids was similar to that of the mucilage but their parameters showed differences. Solutions of PM and DM showed values of k lower than those of XG and GG. However, solutions of PM and DM at 4.5% (w/v) showed a value of k comparable with that of GG solution at 1.5% (w/v).

The flow behavior index (n) corresponding to the PM and DM curves shown in Table 3 was higher than for GG solutions, which means that the mucilage is more resistant to

Table 4 Viscosity and normal force coefficient PM and DM solutions in the absence and presence of ions

	PM	PM NaCl	PM KCl	PM $CaCl_2$	PM $MgCl_2$
η (Pa s)	1.2	0.09	0.1	0.1	0.15
Ψ_1 normal stress coefficient	0.09	0.01	0.01	0.02	0.02
	DM	DM NaCl	DM KCl	DM $CaCl_2$	DM $MgCl_2$
η (Pa s)	0.08	0.07	0.07	0.11	0.15
Ψ_1 normal stress coefficient	0.02	0.008	0.007	0.02	0.03

Data at shear rate = 100 s^{-1}

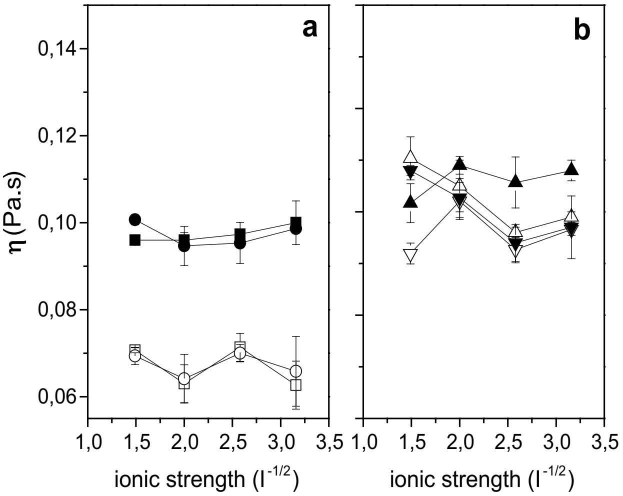

Fig. 3 Apparent viscosity versus ionic strength. **a** Monovalent ions filled circle Na^+, filled square K^+ for PM.; open circle Na^+, open square K^+ for DM, **b** divalent ions filled triangle Mg^{++}, filled inverted triangle Ca^{++} for PM; open triangle Mg^{++}, open inverted triangle Ca^{++} for DM. Data at shear rate = 100 s^{-1}

increasing shear rate. XG showed the lowest *n* value, which indicates a very strong dependence of viscosity on the shear rate, characteristic of the rigid structure of this molecule [43].

Although at low concentrations mucilage is less viscous, at high concentrations it has a similar behavior to that of XG and GG. It was also observed that the mucilage is more resistant to shear rate than guar and xanthan gums.

Influence of electrolytes on the viscosity

The apparent viscosity values of PM and DM aqueous solution at 1.5% (w/v), taken from flow curves (not shown) at a shear rate of 100 s^{-1}, were 1.2 and 0.08 Pa s, respectively (Table 4). The viscosity of the PM solution was about 15 times greater than that of the DM. This could be related to the higher intrinsic viscosity of the sample.

The addition of mono- and divalent ions to the aqueous solutions of PM and DM reduced their apparent viscosity, as shown in Fig. 3. This behavior is characteristic of negatively charged polyelectrolytes. The molecule adopts a less expanded conformation due to the screening effect of the positive ions, which reduce the strong intermolecular repulsions and consequently, the viscosity. This may explain the high viscosity of PM in deionized water; the repulsion between charged sites prevails, causing the chain to adopt an extended conformation [12]. The viscosity value decreased 10 times for PM and only slightly for DM when monovalent ions were added, which could be a result of the less expanded molecule and fewer number of carboxylic groups exposed by DM. These results reinforce the lower molecular weight, less flexibility and fewer negative exposed groups of DM with respect of PM.

When divalent ions were added, an increase in the viscosity of DM and no changes in PM were observed. Taking into account that the available negative groups in the DM are on the surface, the increase of the viscosity in the presence of the divalent ions would be the results of aggregate or net formation. Trachtenberg and Mayer [20] found that Ca^{2+} can be reabsorbed under conditions of calcium deficiency, and Majdoub et al. [12] reported that the increase of viscosity could be interpreted as the result of complex formation between the calcium ions and the carboxylic groups present. In this sense, the first coefficients of normal stress (ψ_1) given in Table 4 describe the viscoelastic behavior, which would imply the possible formation of a network. These parameters were the highest for the PM samples, showing a small and not significant increase in the presence of divalent ions with respect to the monovalent ones. Furthermore, the ψ_1 values of DM in the presence of Ca^{2+} and Mg^{2+} were higher than those found in the presence of monovalent ions and are comparable to those shown by PM in the presence of divalent ions. This would indicate an increase in elastic behavior due to the formation of a macromolecular network when the dialysate mucilage molecule reabsorbed divalent ions.

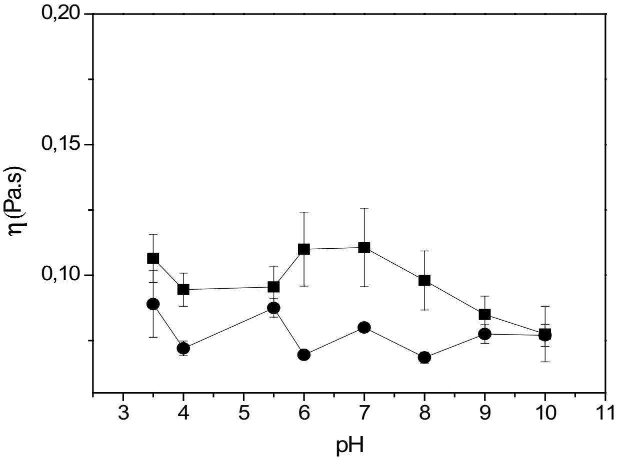

Fig. 4 Apparent viscosity versus pH filled square PM; filled circle DM. Data at shear rate = 100 s^{-1}

Another interesting result found in this work is that after this decrease of viscosity due to the additions of ions, the viscosity remained constant in the range of ionic strength (I) 0–0.6 M applied, meaning that the polyelectrolyte character was minimized in this domain. The cation concentrations used in this work to achieve the desired ionic strengths were greater than the 10 mM value found as critical concentration by Trachtenberg and Mayer [5], upon which a constant viscosity is obtained in mucilage solution probably due to the neutralization of the acid groups.

Influence of pH on the viscosity

Figure 4 displays the influence of pH on the steady flow viscosity (at the shear rate of 100 s^{-1}) of 1.5% (w/v) PM and DM solutions. The viscosity remains almost constant at a pH range of 3–10 for both samples. This result was different to that informed by Majdoub et al. [12], who measured the viscosity by varying the pH in a range of 2–11 while maintaining the ionic strength constant (0.025 M). They found that on raising the pH, the viscosity increased sharply in the pH range 4–6, indicating a conformational transition from a compact to a highly solvated structure. This is explained by the mutual repulsion of the fixed charges involving the swelling of macromolecules. In contrast, in our study, the buffers used to adjust pH values produced varying ionic strengths in the range of 0.1–0.5 M, which was much higher than the critical point suggested by Trachtenberg and Mayer [5]. Under these conditions, the mucilage charges would be completely screened; therefore, changes in pH did not significantly modify their viscosities. At high ionic strength,

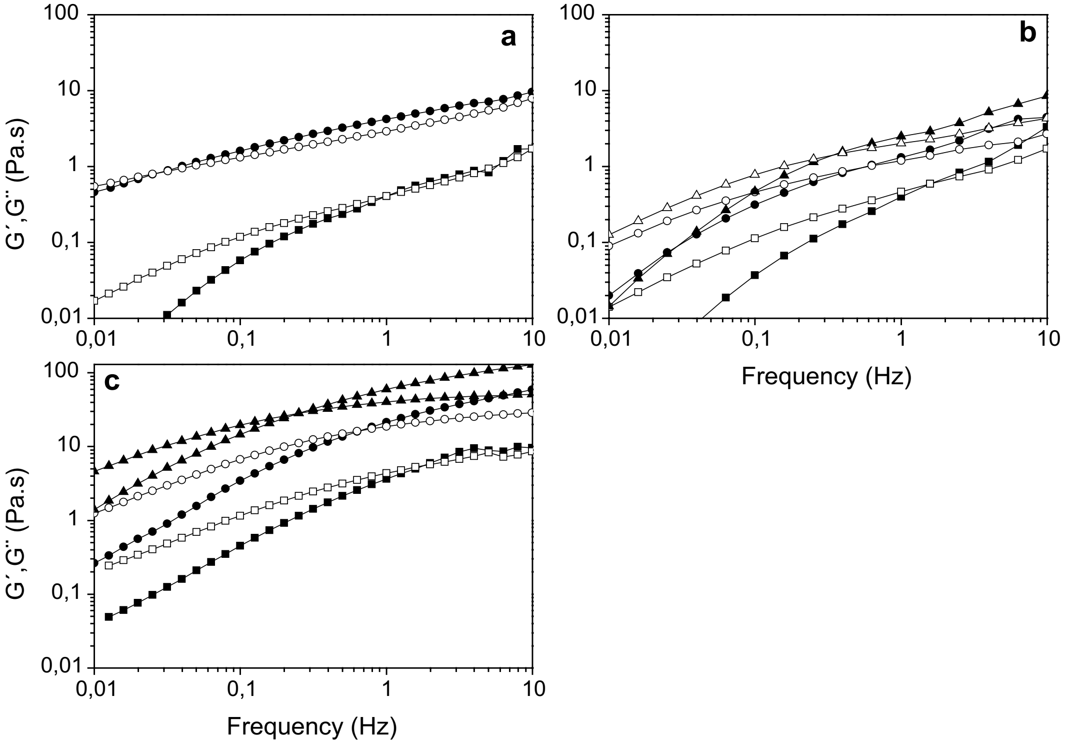

Fig. 5 Frequency dependence of the storage modulus G′ and loss modulus G″ for different concentrations (% w/v) filled square 0.5, filled circle 1, filled triangle 1.5 of PM (**a**), DM (**b**) and GG (**c**). Filled symbols, G′. Blank symbols, G″

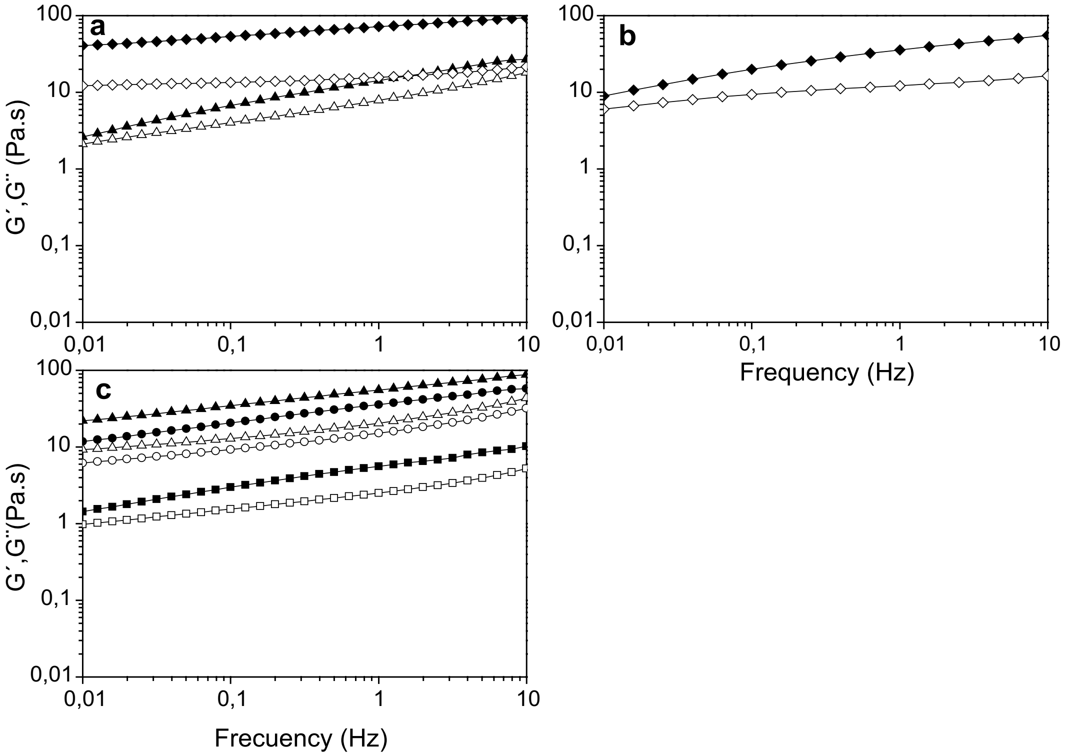

Fig. 6 Frequency dependence of the storage modulus G′ and loss modulus G″ for different concentrations (% w/v) filled square 0.5, filled circle 1, filled triangle 1.5, filled diamond 4.5 of PM (**a**), DM (**b**) and XG, (**c**). Filled symbols, G′. Blank symbols, G″

the region of sharp viscosity change disappeared due to the salt screening of chain charges, which caused the chain to coil up [12]. It is important to know that in this work it was necessary to use buffers for preparing solutions, because the mucilage showed strong buffering effects, attributable to the presence of acid–base substances, as Corrales-Garcia et al. [44] noted.

Considering the behavior of the other hydrocolloids, the viscosity of xanthan gum remains stable at low pH [45], while galactomannan [46] and guar gum [47] can degrade and lose viscosity at high or low pH.

Steady oscillatory flow measurements

Figures 5 and 6 show the mechanical spectra describing the viscoelastic behavior of PM and DM solutions at different concentrations. Both the dynamic storage modulus (G′) and the viscous modulus (G″) of the PM and DM solutions showed dependency on the frequency. This behavior is characteristic of an entangled network of disordered polymer coils [17].

As illustrated in Fig. 5 a, at low concentration (0.5% w/v), the viscous modulus G″ of PM was higher than G′ at the low frequency region, until a certain characteristic frequency ω_c, where a crossover of the G′ and G″ versus frequency curves occurred $[G'(\omega_c) = G''(\omega_c)]$. After that, the behavior became rather elastic ($G' > G''$) [48]. This crossover suggests a conformational change in the molecule and has been explained as the transition from a random coil to an entangled conformation [49]. The mechanical spectrum of the 1% (w/v) PM solution showed its crossover at lower frequencies (Fig. 5.a), which implies a higher elastic contribution [50].

The 0.5, 1 and 1.5% (w/v) DM solutions (Fig. 5b) showed a similar behavior to that of PM samples with a crossover point on the mechanical spectrum that occurred at higher frequency.

Furthermore, their G′ and G″ moduli were lower than those of PM, as were their intrinsic viscosity (Table 2) and apparent viscosity (Table 4).

The same experiments were carried out with the gums used as control. The mechanical spectrum of GG studied at 0.5, 1 and 1.5% (w/v) showed a crossover (Fig. 5c), which shifted to lower frequencies with increasing concentration. This means that the rheology of polysaccharide solutions is governed mainly by the degree of entanglement of individual macromolecules, which is characteristic of entangled polymer solutions [28]. Similar results have been reported by Oblonsek et al. [51] and Sittikijyothin et al. [52] for guar gum and other galactomannans.

The relaxation time $\lambda\left(\lambda = 1/\omega_c\right)$ can be estimated from the ω_c frequency value at the crossover point and represents the tendency of the material to flow [53]. Table 3 lists the relaxation times (λ) obtained from the frequency rheograms of PM, DM and GG. The relaxation times of PM and DM samples were higher than those of GG. An increase in relaxation time was also observed when gum concentration increased. Relaxation times in the order of 0.1 s indicated low elasticity and those in the order of 100 s were related to a marked increment in the elastic behavior and the strength of the structure [54]. The value of 50 s for the 1% (w/v) PM sample stands out in relation to that of 1.4 s corresponding to GG at the same concentration. The difference between both might be associated with the occurrence of high density entanglements or aggregates in the mucilage. The building macromolecule of the mucilage responds more slowly to the deformation applied, needs more time to reorganize its structure, and reaches a new equilibrium state after an external stress has been applied.

On the other hand, another kind of behavior was observed at the highest concentrations of PM and DM. The mechanical spectra of the solutions at 1.5, 4.5% for PM and 4.5% (w/v) for DM (Fig. 6a, b) showed viscoelastic behavior with G′ predominating over G″ in the experimental range of frequencies. The G′ and G″ moduli of PM and DM solutions at 4.5% (w/v) showed less frequency dependence. This behavior indicates a clear tendency to form macromolecular networks with important elastic properties rather than physical gel characteristics where G′ and G″ are independent of frequency [55]. Medina-Torres et al. [56] found that the mucilage is a polysaccharide that cannot form gels by itself, but has a synergistic effect in the *k*-carrageenan gels giving a stronger structure.

The mechanical spectra of PM and DM at higher concentration were similar to the spectra of xanthan gum solutions (Fig. 6c) in which G′ was higher than G″ along the entire range of frequencies and at all the concentrations studied. The xanthan molecule adopts a helical conformation that is more rigid [57], implying a much more limited chain mobility and therefore, much longer relaxation time. At low concentration, the mucilage solution had a similar behavior to that of guar gum solution, but at high concentration it was similar to that of xanthan gum solution. This behavior might be related to the existence of macromolecular networks and the formation of aggregates when the concentration increases.

The Cox-Merz rule

The Cox-Merz rule is an empirical relationship between dynamic viscosity and shear viscosity [58].

$$\eta^*_{(\omega)} = \eta_{(\gamma)|\omega=\dot{\gamma}|} \tag{5}$$

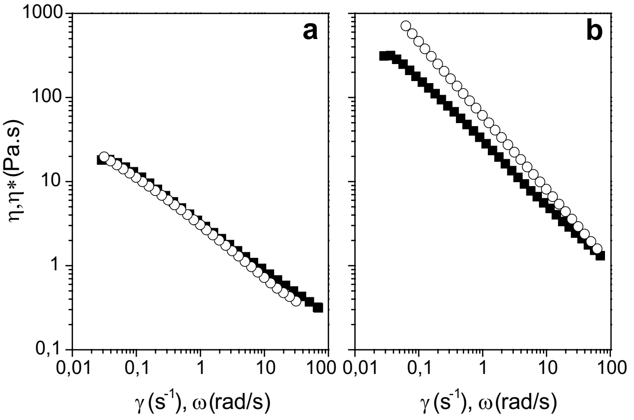

Fig. 7 Open circle complex viscosity [η*(ω)] and filled square apparent viscosity [η(γ)] of PM solutions: **a** 1.5% (w/v), **b** 4.5% (w/v)

According to this rule, the two parameters superimpose at equal values of frequency and shear rates if the polymer solution is devoid of energetic interactions. This rule has been confirmed experimentally for most of the random-coil biopolymer solutions. In contrast, solutions of polysaccharides with a rigid and ordered chain conformation, or with high density entanglement or aggregates showing a structured liquid behavior (e.g., cress seed gum, xanthan, gellan and schizophyllan polysaccharides) violate the Cox-Merz rule. In this case, the dynamic complex viscosity is higher than the shear viscosity at equivalent rates of deformation, indicating a tenuous network that remains intact under low amplitude oscillation but is disrupted under continuous shear [59].

PM and DM solutions at low concentrations (0.5 and 1%) met the Cox-Merz rule, which would indicate that under these conditions the molecule presents a random coil conformation. Similarly, GG solutions at these same concentrations showed no deviations from this rule (data not shown).

More concentrated PM and DM (1.5 and 4.5%) and GG (1.5%) solutions showed deviation from the Cox Merz rule. Figure 7 shows the relationship between the dynamic viscosity and shear viscosity data obtained for the PM sample. In the more concentrated solution [η]* was higher than $\eta_{app,}$ indicating disruption of the PM structure under continuous shear rates and violating the Cox-Merz rule. In this sense, Cárdenas et al. [17] reported aggregate formation through SECHPLC studies. DM solutions exhibited the same behavior as that of PM but with lower viscosities (data not shown).

The xanthan gum solution showed deviations of the rule at all the concentrations studied. Wang and Cui [31] attributed this deviation to weak intermolecular associations among xanthan gum molecules, which leads to the formation of a three-dimensional network structure that can break easily even at a very low.

Conclusions

This study has shown that mucilage presented high intrinsic viscosity, with similarities in the properties studied to guar gum and xanthan gum. Mucilage exhibited shear-thinning behavior with viscosities lower than those of GG and XG at the same concentration. The pH and ionic strength not affected the viscosity under the experimental conditions. The viscoelastic properties of mucilage were similar to those of GG at lower concentrations and to those of XG at higher concentrations. A particular result was the high relaxation time of mucilage indicating that the mucilage responds more slowly to the deformation applied than GG and XG.

Mucilage exhibited the ability to reduce the surface tension of water and it was concentration dependent and the electrophoretic measurement indicated that it is a negatively charged polyelectrolyte under the test conditions.

Mucilage has promising properties to be used in many technological applications, such as stabilizer or thickener in food formulations, in biofilms or wall component in capsules that can be easily extracted from a natural source.

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