# Rheological characterization of the hydrocolloid from *Gleditsia amorphoides* seeds

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## ARTICLE INFO

## ABSTRACT

Espina Corona Gum (ECG) is extracted from *Gleditsia amorphoides* seeds and is used as an additive in Argentinean food industry. Its chemical structure corresponds to a galactomannan. ECG was compared with Guar Gum (GG) as regards several properties, among them those related to rheological behavior, the effect of NaCl concentration, temperature and acidity. Experimental results demonstrated that ECG solutions have a shear-thinning behavior, respond to a power-law model, are influenced by temperature, and show a good stability when heated. The presence of NaCl and acidity did not affect ECG solution viscosity. ECG solutions were less viscous and less shear-thinning than GG ones. The viscoelastic behavior shows that, for low frequencies, the viscous modulus is greater than the elastic one up to the crossover point of the frequency, where this behavior is reverted. The apparent viscosity decreased as the frequency of oscillation increased.

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## 1. Introduction

Hydrocolloids are hydrophilic high molecular weight biopolymers which have the ability to greatly hydrate in contact with water, thus producing colloidal systems of different structures, and which significantly increase the system viscosity even at low concentrations (Vardhanabhuti & Ikeda, 2006). Because of these properties, hydrocolloids are used as food additives to obtain particular textural characteristics (Amina, Ahmad, Yap, Norfariza, & Norhayati, 2007; Glicksman, 1982). They are broadly used in food systems as thickeners, gelling agents, texture modifiers, suspending agents, and stabilizers (Chaubey & Kapoor, 2001; De Man, 1999). Plant hydrocolloids are beneficial to consumers because of their friendly image toward the environment (Vardhanabhuti & Ikeda, 2006). Galactomannans are non-gelling polysaccharides commercially important mainly for their thickening properties. The two galactomannans of utmost commercial importance are Guar Gum (GG), from *Cyamopsis tetragonolobus*, and Locust Bean Gum (LBG), from *Ceratonia siliqua* (Dakia, Blecker, Roberta,

Watheleta, & Paquota, 2008). Espina Corona Gum (ECG) is a galactomannan extracted from the seeds of Espina Corona (*Gleditsia amorphoides*), a leguminous tree native of Latin America that grows in the forests of Northern Argentina. It was grown in Argentina in the 50's and 60's (Riqué & Pardo, 1952; pp. 1—28); then its production was discontinued, although it never ceased completely. Today, due to the increasing cost of similar foreign products, Espina Corona (EC) production is being revitalized through various businesses. However, despite the rich flora biodiversity and the favorable climate for their production, galactomannans from Latin American sources are not well known (Azero & Andrade, 2002).

Galactomannans are neutral polysaccharides consisting of a linear mannose backbone bearing side chains of a single galactose unit (Azero & Andrade, 2002). The chemical composition of ECG was postulated by Cerezo in 1965 as a galactomannan structure composed of 71.4 g/100 g D-mannose and 28.6 g/100 g D-galactose with a mannose-to-galactose (M/G) ratio of 2.5 (Cerezo, 1965). The mannose forms a linear chain of $(1 \rightarrow 4)$ β-mannopyranose units with one molecule of D-galactopyranose linked at position 6 every three units of mannose. This relation is very similar to that of other galactomannans and already well-known for galactomannans such as Guar Gum, with 1 galactose every 2 units of mannose and 2.0 M/G ratio (Chaubey & Kapoor, 2001; Doublier & Launay, 1981; Whistler & BeMiller, 1997).

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The gum's usual purification processes include its extraction from the seeds using water as a solvent, followed by filtration or centrifugation. The gum can be recovered by precipitation with alcohol, drying in an oven and milling, or freeze-drying or spray-drying (Azero & Andrade, 2002; Ibañez & Ferrero, 2003; Oomah & Mazza, 2001). In general, the purity of ECG commercial products is not too high in the Argentinean market. The gum is not often commercially available in purified forms and contains significant amounts of insoluble fraction. This insoluble fraction reduces the product value since it renders the gum inappropriate to be used in food formulations that need clear shades or light colors. Instead, the product has black spots from the tegumental residues that cover the seed's endosperm. These black spots are generated in the roasting procedure used to remove the tegument.

The composition of ECG plays an important role in its rheological properties, and the rheological behavior also depends on the method used to obtain the gum (Azero & Andrade, 2002).

The objectives of this research were to study a gum purification process applicable in industry, using a dryer to remove impurities and insoluble fraction, as well as to study the chemical composition and the rheological properties of the gum. With this purpose, the effects of concentration, temperature, sodium chloride and glucono-δ-lactone (GDL) on the flow behavior of polysaccharides were investigated.

## 2. Materials and methods

### 2.1. Materials

The industrial process of ECG carried out by Idea Supply Argentina S.A. (Chaco, Argentina) consists of washing the seeds in chlorinated water, toasting at 120 °C during 1 h, and shelling with grinding equipment. Subsequently, the seeds are sifted to separate the shells and finally they are ground. The ECG flour (ECGF) obtained has light cream thin granules with small dark particles derived from the tegument, and is sold as such.

Guar Gum (GG) is a marketing sample provided by Sapporiti S.A. (Buenos Aires, Argentina).

### 2.2. Purification of the Espina Corona Gum flour

ECGF (20 kg m$^{-3}$) was suspended in tap water and stirred at 60 °C for 3 h. Later, it was filtrated with an ASTM 40 (420 μm) sieve, thus obtaining a solution from the gum and a retained material composed of tegumental residues and undissolved fractions. Dry solids were determined by weight loss in an oven at 105 °C for both fractions. Subsequently, the gum solution was spray-dried at a pilot plant with a Niro atomizer spray-dryer (Denmark) using an inlet temperature of 200 °C and an outlet temperature of 90 °C.

### 2.3. Chemical analysis

Moisture, crude fat, total protein, crude fiber and ash contents were determined according to the approved AOAC International 934.01, 920.39, 2001.11, 942.05, 962.09 (AOAC, 2006), respectively. All trials were conducted in triplicate.

### 2.4. Color analysis

The color of both powder and solution was evaluated with a Minolta CM-508d colorimeter (Japan) using a D65 illuminant and a 10° observer angle. The samples were compressed for 5 s with a weight of 1 kg. The color was expressed in terms of lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$).

### 2.5. Rheological characterization

#### 2.5.1. Determination of intrinsic viscosity

Determination of intrinsic viscosity $[\eta]$ was carried out by extrapolation of the Huggins and Kramer model, expressed by equations (1) and (2), respectively (Mothé & Rao, 1999), considering the relative viscosities $[\eta_{rel}]$ within the 1.2−2.0 range.

$$\frac{\left[\eta_{sp}\right]}{C} = [\eta] + k_1 \cdot [\eta]^2 \cdot C \tag{1}$$

$$\frac{\ln[\eta_{rel}]}{C} = [\eta] + k_2 \cdot [\eta]^2 \cdot C \tag{2}$$

where $[\eta_{sp}]$ is specific viscosity.

The viscometric average molecular masses $\overline{M}_v$ were calculated using the Mark−Houwink equation (3) given by Doublier and Launay (1981) for Guar Gum and modified by Gaisford, Harding, Mitchell, and Bradley (1986) to take into account the different M/G values in galactomannans:

$$\left[\eta\right] = 11.55 \cdot 10^{-6} \left[(1-r)\overline{M}_v\right]^{0.98} \tag{3}$$

where $r$ is the galactose/(mannose + galactose) ratio and $[\eta]$ is the intrinsic viscosity, in dL g$^{-1}$.

#### 2.5.2. Rotational rheological analyses

A 20 kg m$^{-3}$ sample of an ECG solution was prepared, left overnight at room temperature, and centrifuged at $1800\times g$ for 1 h. The supernatant was used for further dilutions. Rheological measurements were carried out with an HAAKE RheoStress RS80 (Haake Mess − Technik GmbH., Germany), with a plate-and-plate geometry of 35 mm diameter and 1 mm of gap between the plates, varying the shear rate ($\dot{\gamma}$) from 0.1 to 1000 s$^{-1}$ for rotational trials. For temperature control, an HAAKE N2T bath was used and the data were analyzed with the HAAKE RheoWin 3.50.0011 software. All trials were conducted in triplicate.

The shear-thinning behavior of fluids can be modeled in a limited interval of shear rate ($\dot{\gamma}$) with Ostwald's power law (Rosenthal, 2001; pp. 69−80) equation (4).

$$\eta_{ap} = k\dot{\gamma}^n \tag{4}$$

being $\eta_{ap}$ the apparent viscosity (Pa s), $k$ the consistency coefficient (Pa s$^n$), $\dot{\gamma}$ the shear rate (s$^{-1}$) and $n$ the flow behavior index (dimensionless).

##### 2.5.2.1. Effect of gum concentration.
The effect of gum concentration on $\eta_{ap}$ was studied preparing ECG solutions at concentrations of $15 - 10 - 7.5 - 5.0 - 2.5$ kg m$^{-3}$. Measurements were carried out at $25 \pm 0.1$ °C. ECG solutions were compared with GG solutions at $25 \pm 0.1$ °C at 5.0 and 10 kg m$^{-3}$.

##### 2.5.2.2. Effect of temperature.
The effect of temperature (10, 25, 40 and $60 \pm 0.1$ °C) on the $\eta_{ap}$ of 10 kg m$^{-3}$ ECG solution was determined.

The hydrocolloid stability after heating was determined on a 5.0 kg m$^{-3}$ ECG solution. First, the $\eta_{ap}$ of the ECG solution was measured at 25 °C; the solution was then heated for 30 min at different temperatures (60 °C, 75 °C and 90 °C), and finally the temperature was reduced to $25 \pm 0.1$ °C and $\eta_{ap}$ was measured again.

*2.5.2.3. Effect of NaCl concentration.* ECG solutions of 5.0 kg m$^{-3}$ were prepared, adding then sodium chloride at different concentrations (0, 30, 60 and 90 mM) and determining their $\eta_{ap}$ at $25 \pm 0.1$ °C.

*2.5.2.4. Effect of pH.* ECG solutions at 5.0 kg m$^{-3}$ were prepared and the effect of gluco-δ-lactone (GDL) concentration ($0 - 0.5 - 1.5 - 3.0 - 6.0$ g l$^{-1}$) was tested. Their $\eta_{ap}$ was determined at $25 \pm 0.1$ °C as well as their final pH. The effect of adding GDL to 7.5 kg m$^{-3}$ ECG solutions on $\eta_{ap}$ was also analyzed after heating at 75 °C for 30 min.

### 2.5.3. Oscillatory dynamic rheological analyses

For dynamic rheological trials the HAAKE RheoStress RS80 rheometer was used, with a plate-and-plate geometry of 35 mm diameter and 1 mm of gap between the plates. Varying the frequency ($f$) from 0.010 to 1.500 Hz with a 4% constant strain in the linear viscoelastic range (Ibañez & Ferrero, 2003), measurements of G′ (dynamic elastic modulus), G″ (dynamic viscous modulus) and $\eta^*$ (complex viscosity) were obtained to determine the mechanical characteristics of the samples. All measurements were carried out at $25 \pm 0.1$ °C, controlled under an HAAKE N2T bath, and data were analyzed with an HAAKE RheoWin 3.50.0011 software. All measurements were conducted in duplicate.

## 3. Results and discussion

### 3.1. Characterization and chemical composition

The purification process implemented in this paper is used at an industrial level; however, other methods are preferred in laboratory experiments (Ibañez & Ferrero, 2003; Wielinga & Maehall, 2000).

The composition of the ECGF and the purified ECG samples is shown in Table 1. The soluble and insoluble fractions of the ECGF sample were determined, which shows that after solubilization, 30.5 g/100 g of the sample remains insoluble, and the rest (69.5 g/100 g), composed of the gum solution, appears translucent and free of tegumental residues. The samples are mainly composed of polysaccharides. Lipid concentration in ECGF is very low and it is halved in the ECG sample. Protein determination showed a decrease of 46% for the ECG sample, whereas ashes slightly increased for ECG as compared with ECGF.

Ideally, gums should be colorless to prevent any color changes in food products upon their addition. ECG shows high lightness ($L^* = 85.43 \pm 0.59$) and low yellowness ($b^* = 8.61 \pm 0.08$), resulting in significant difference with the ECGF ($p \leq 0.05$). Redness contribution ($a^* = -0.01 \pm 0.02$), on the other hand, is low both in ECG and in ECGF, with no significant difference ($p \leq 0.05$). These results indicate that ECG has good quality in terms of color and a potentially good applicability, similar to that of the GG sample, with $L^* = 84.83 \pm 0.01$, $a^* = -0.45 \pm 0.01$ and $b^* = 12.94 \pm 0.06$.

### 3.2. Intrinsic viscosity

The intrinsic viscosity $[\eta] = 8.46$ dL g$^{-1}$ was determined according to Huggins' equation (1), and $[\eta] = 8.90$ dL g$^{-1}$ was

determined according to Kramer's equation (2), respectively. Averaging the values obtained by equations (1) and (2), and using the M/G ratio = 2.5 (Cerezo, 1965), the viscometric average molecular weight (calculated by equation (3) using the previous intrinsic viscosity values obtained) was $\overline{M}_v = 1.39 \cdot 10^{-6}$. This value is lower than that reported by Azero and Andrade (2006) for GG $\overline{M}_v = 1.81 \cdot 10^{-6}$.

### 3.3. Rotational rheological trials

The effect of ECG concentration on the flow behavior of the solutions is shown in Fig. 1. It can be observed that solutions show a pseudo-plastic or shear-thinning behavior (Rao, 1999). Under the working conditions and concentrations higher than 5.0 kg m$^{-3}$, ECG solutions would fit the power-law model. However, at concentrations of 2.5 kg m$^{-3}$ the linearity remains only in the initial area and, as shear rate ($\dot{\gamma}$) increases to $3.5 \cdot 10^2$ s$^{-1}$, the solutions tend to be Newtonian. This behavior is due to the fact that when these particles are at rest they are arranged in no particular order, which causes a great internal resistance to flow. As shear rate increases, the particles are flow-oriented, which decreases slip resistance and therefore the apparent viscosity (Vardhanabhuti & Ikeda, 2006).

The values of flow behavior index ($n$) and consistency coefficient ($k$) obtained through the power law (Equation (4)) are shown in Table 2. The increase in ECG concentration raises both pseudo-plasticity and viscosity, which results in an increase in $k$ and a decrease in $n$.

Comparing trials between ECG and GG at an equal concentration, GG shows higher $\eta_{ap}$ values than ECG. This would be associated to the higher molecular weight of GG as compared with that of ECG (Williams & Philips, 2000). Regarding the constants in the power-law model, the consistency coefficient increases with concentration for both gums, while the flow behavior index decreases (Table 2).

Fig. 2 shows the effect of temperature on the $\eta_{ap}$ of 10 kg m$^{-3}$ ECG solutions. It can be observed that when temperature increases the apparent viscosity (lower $k$) decreases. This behavior suggests that the higher the temperature, the less pseudo-plastic solutions are, due to higher flow behavior index (Table 2). Generally, galactomannan solutions show a 50% decrease in viscosity when temperature rises from 20 °C to 80 °C (Wielinga & Maehall, 2000).

The stability study of ECG after heating was studied for different temperatures (60, 75 and 90 °C). Like several hydrocolloids such as GG and Xanthan Gum, the ECG solutions were not affected by the high temperatures (Downey, 2002).

The effect of NaCl, which modifies the environment's ionic strength, on the viscosity of the ECG solutions, was studied. Charged polymers are known to have a viscosity higher than that of non-ionic polymers with similar molecular mass because their molecular chains expand due to the repulsions of intramolecular charges. The addition of electrolytes reduces the degree of dissociation of the charged groups, which usually leads to chain compaction and a significant decrease in viscosity (Williams & Philips, 2000). In this case, since ECG is not a charged polymer, there are not any changes in the apparent viscosity of solutions at

**Table 1**
Composition of ECG samples (g/100 g on dry basis).

| Sample | Moisture (g/100 g) | Ash (g/100 g) | Crude fat (g/100 g) | Total protein (g/100 g) | Crude fiber (g/100 g) | Carbohydrate[a] (g/100 g) |
|---|---|---|---|---|---|---|
| ECGF | 6.93a ± 0.08 | 1.02a ± 0.01 | 0.61a ± 0.05 | 3.99a ± 0.11 | 1.71a ± 0.27 | 85.74 |
| ECG | 10.04b ± 0.24 | 1.44b ± 0.04 | 0.27b ± 0.01 | 2.17b ± 0.01 | 0.70b ± 0.23 | 85.38 |

The values represent the mean ± standard deviation of triplicate tests. Different letters mean significant differences ($p \leq 0.05$).
  [a] Calculated by difference.
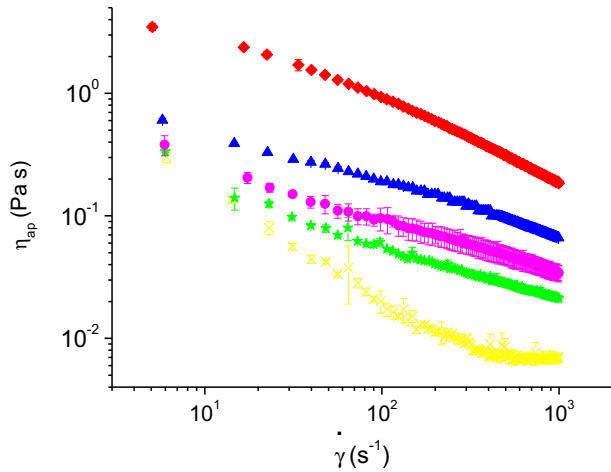
**Fig. 1.** Flow behavior of ECG solutions at different concentrations: (✕) 2.5 kg m$^{-3}$, (★) 5.0 kg m$^{-3}$, (●) 7.5 kg m$^{-3}$, (▲) 10 kg m$^{-3}$, (◆) 15 kg m$^{-3}$.
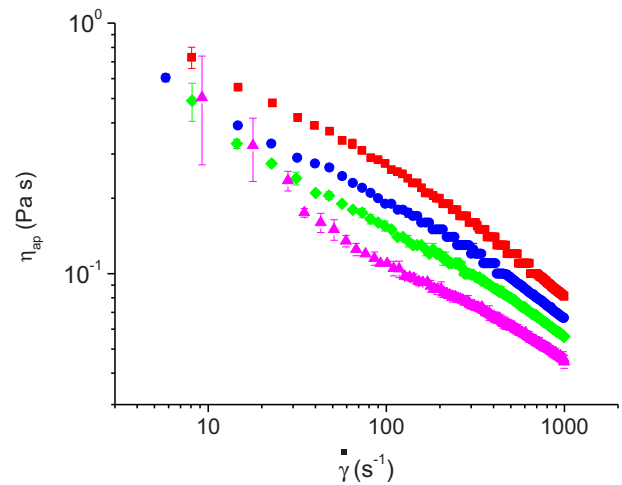


**Fig. 2.** Flow behavior of ECG 10 kg m$^{-3}$ at different temperatures: (■) 10 °C, (●) 25 °C, (◆) 40 °C, (▲) 60 °C.

different concentrations of NaCl when compared with the solution without this salt (results not shown). Similar results were found for galactomannans such as GG or Locust Bean Gum, the viscosity of which is not influenced by the addition of electrolytes.

The effects of pH on hydrocolloid viscosity vary with the hydrocolloid species. The Xanthan Gum maintains its viscosity and stability at low pH (García-Ochoa, Santos, Casas, & Gómez, 2000) while carboxymethylcellulose (CMC) and some galactomannans show a decrease in viscosity when pH decreases (Glicksman, 1982). To study this phenomenon in ECG solutions, the addition of GDL was tested, which slowly reduces the pH through its hydrolysis. The pH of a 5.0 kg m$^{-3}$ ECG solution without GDL is 6.60. The trials with GDL show that the change in pH does not affect the apparent viscosity of these solutions (results not shown).

Galactomannans subjected to high temperatures could be broken down at high or low values of pH (Williams & Philips, 2000) though this was not found for 5.0 kg m$^{-3}$ ECG solutions, which maintain their behavior after being heated at 75 °C for 30 min (Fig. 3).

ECG viscosity remains unchanged when the ionic strength varies, and the final pH indicates that this hydrocolloid does not have charged groups that could vary the electrolytic behavior of the solution (Williams & Philips, 2000). These effects are important in the application of ECG in emulsions and foams since its viscosity will not undergo variations when the concentration of salts and/or the pH of food matrixes are modified.

### 3.4. Oscillatory dynamic rheological trials

The viscoelastic behavior was analyzed in 5.0 kg m$^{-3}$ and 10 kg m$^{-3}$ ECG solutions at a constant strain ($\gamma$) of 4%, the mechanical spectrum being shown in Fig. 4a. Since ECG is a hydrocolloid that produces viscoelastic solutions, it could be characterized by the magnitude of the dynamic storage ($G'$) and the loss modulus ($G''$) and their respective dependence on the oscillation frequency. In the diluted solutions, $G''$ is higher than $G'$ in a large portion of the frequency range where the intermolecular disorder does not take place (Williams & Philips, 2000). At higher concentrations, $G'$ and $G''$ are even more dependent on frequency. The viscous modulus, $G''$, is higher than the elastic modulus, $G'$, up to the crossover point of frequency, showing values of 1.0 Hz for an ECG concentration of 5.0 kg m$^{-3}$ and 1.5 Hz for 10 kg m$^{-3}$. The complex viscosity ($\eta^*$) associated with the overall viscoelastic behavior decreases in terms of frequency. These results show the dependence of viscoelastic behavior on the concentration of the solution.

The frequency sweep (mechanical spectrum) shows a typical behavior for macromolecules solutions: at low frequencies, the loss

**Table 2**
Comparison between the flow behavior index ($n$) and the consistency coefficient ($k$) for ECG and GG at different temperatures.

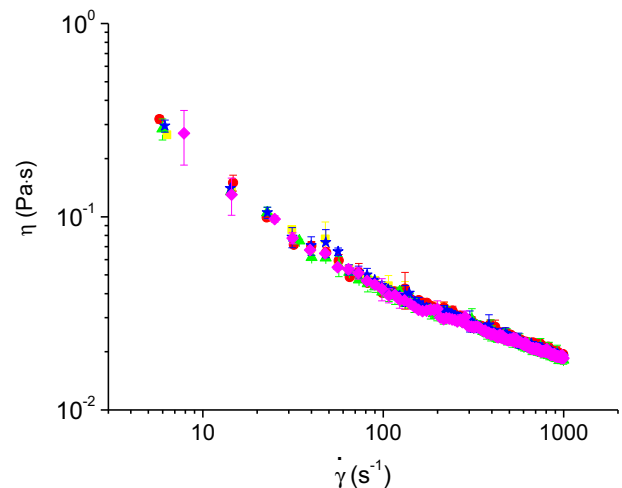| Sample | Concentration (kg m$^{-3}$) | Temperature (°C) | $k$ (Pa s$^n$) | $n$ (dimensionless) | $R^2$ |
|--------|------------------------------|------------------|-----------------|----------------------|-------|
| ECG | 2.5 | 25 | $0.254 \pm 0.031$ | $0.444 \pm 0.023$ | 0.897 |
|  | 5.0 | 25 | $0.409 \pm 0.033$ | $0.570 \pm 0.015$ | 0.981 |
|  | 7.5 | 25 | $0.650 \pm 0.070$ | $0.578 \pm 0.002$ | 0.994 |
|  | 10 | 10 | $2.747 \pm 0.016$ | $0.495 \pm 0.001$ | 0.989 |
|  |  | 25 | $1.567 \pm 0.021$ | $0.548 \pm 0.002$ | 0.991 |
|  |  | 40 | $1.089 \pm 0.020$ | $0.576 \pm 0.005$ | 0.995 |
|  |  | 60 | $0.692 \pm 0.004$ | $0.609 \pm 0.003$ | 0.978 |
|  | 15 | 25 | $20.717 \pm 1.135$ | $0.323 \pm 0.006$ | 0.988 |
| GG | 5.0 | 25 | $1.827 \pm 0.056$ | $0.383 \pm 0.004$ | 0.999 |
|  | 10 | 25 | $50.599 \pm 6.217$ | $0.139 \pm 0.001$ | 0.999 |

The values represent the mean ± standard deviation of duplicate trials.



**Fig. 3.** Effect of GDL on the apparent viscosity at 25 °C for a 5.0 kg m$^{-3}$ ECG solution heated at 75 °C for 30 min: (■) 0 g/100 g GDL, pH = 6.60; (●) 0.05 g/100 g GDL, pH = 4.70; (▲) 0.15 g/100 g GDL, pH = 4.34; (★) 0.30 g/100 g GDL, pH = 4.03; (◆) 0.60 g/100 g GDL, pH = 3.20.
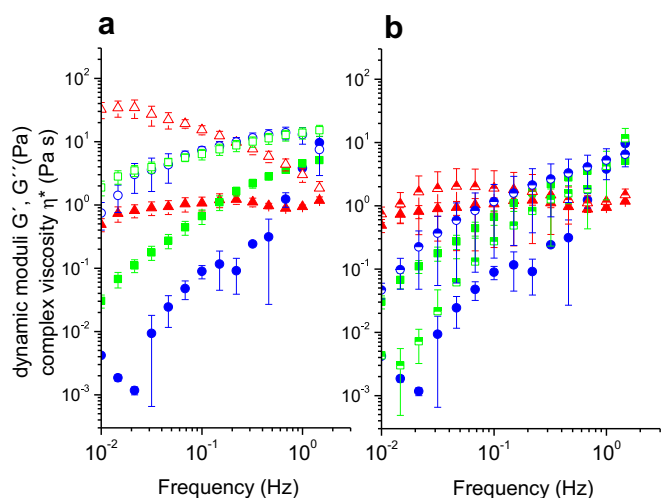
**Fig. 4.** Mechanical spectra with $\gamma = 4\%$ at 25 °C, dynamic elastic modulus, G′(●); dynamic viscous modulus, G″(■) and complex viscosity, $\eta^*$ (▲). ECG solutions at a concentration of 5.0 kg m$^{-3}$: Open symbols and 10 kg m$^{-3}$: Full symbols (a) and 5.0 kg m$^{-3}$ GG solution: half open and half full symbols (b).

modulus, G″, is higher than the storage modulus, G′, whereas at high frequencies G′ is predominant (Sittikijyothin, Torres, & Goncalves, 2005).

In solution, some polysaccharides like Xanthan Gum have a tendency to undergo a weak intermolecular chain association for the formation of a three-dimensional structured network. Other polysaccharides, such as amylose, carrageenan and gellan gum, can form stable intermolecular regions of association which consequently produce strong gel structures. Hydrocolloid gels are known as "physical gels" because their bonding sites are formed, for example, through physical interaction, hydrogen bridges, hydrophobic associations, cation crosslink, etc., and differ from synthetic polymer gels in that these usually consist of chains of covalently cross-linked polymers (Williams & Philips, 2000). Fig. 4b shows a comparison between the viscoelastic behavior of GG and that of ECG solutions. The crossover point of frequency is lower for both concentrations of GG. In addition, the higher the hydrocolloid concentration, the lower the difference between the values of the elastic modulus, G′, and the viscous modulus, G″, a behavior similar to that obtained from *Prosopis flexuosa* (Ibañez & Ferrero, 2003).

## 4. Conclusion

The purification of the ECGF sample results in a product than can be used in any type of food with appropriate visual characteristics. The composition of the gum extracted from *G. amorphoides* seeds is similar to that of other gums, with a polysaccharide's content greater than 80 g/100 g.

The rotational rheological results experimentally showed that the viscosity of the ECG solutions has a pseudo-plastic behavior and fits the power-law model for concentrations greater than 5.0 kg m$^{-3}$.

Viscosity is influenced by temperature; increases in temperature from 10 °C to 60 °C result in a decrease in viscosity and the pseudo-plasticity of the solutions of this gum. ECG shows stability after heating, like several hydrocolloids such as GG and Xanthan Gum. Therefore, it can be used in food products that require stability when exposed to heating procedures. The presence of NaCl, which modifies the ionic strength of the solution, and the presence of GDL, which reduces the pH, do not affect the apparent viscosity of ECG. At the same concentration, ECG solutions are less viscous and less pseudo-plastic than GG solutions. Regarding the

viscoelastic behavior, the oscillatory dynamic trials suggest that for low frequencies the viscous modulus of this hydrocolloid is greater than the elastic modulus up to the crossover point of frequency, where this behavior is reverted. The overall complex viscosity decreases in terms of frequency.

Results from this paper support the application of this gum to various food systems since it meets the processing conditions and the interaction with other ingredients in order to obtain particular characteristics.

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