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# *Lepidium perfoliatum* seed gum: investigation of monosaccharide composition, antioxidant activity and rheological behavior in presence of salts

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## Abstract

**Background:** In the present study, the effects of NaCl and $CaCl_2$ (0–200 mM) on the rheological properties of *Lepidium perfoliatum* seed gum (LPSG) as a novel potential source of hydrocolloid were investigated. Sugar composition and FTIR analysis were measured to supply more structural information.

**Results:** The results illustrated that LPSG had small amounts of uronic acids (6.65%) and it is likely an arabinoxylan-type polysaccharide (it has 44.66% and 31.99% xylose and arabinose, respectively). The FTIR spectra also revealed that LPSG behaved like a typical polyelectrolyte due to the presence of carboxyl and hydroxyl groups. It was observed that the gum solutions exhibited viscoelastic properties in the presence of NaCl and $CaCl_2$ salts. The tan δ values for all samples were less than 1 but greater than 0.1, exposing the weak gel-like behavior at different ion types and ionic strengths. With increasing salts concentrations, the limiting values of strain mostly increased due to the inter-chain interactions (from 1.46 to 4.61 and from 0.99 to 2.13 for NaCl and $CaCl_2$, respectively). Therefore, the addition of salts increased the stiffness of mucilage solutions in the concentrated regime. The results of frequency sweep tests revealed that storage and loss moduli were increased with increasing ion concentration. This effect was more pronounced for LPSG solutions containing $Ca^{2+}$. Among various models, the model of Higiro1 showed a higher efficiency to evaluate the intrinsic viscosity of LPSG for all co-solutes ($R^2 \geq 0.98$). With increasing the concentration of salts, the intrinsic viscosity of LPSG decreased. Calcium ions had a more diminution effect on intrinsic viscosity than sodium ions.

**Conclusions:** Trying to adjust the salt concentration could modify the rheological properties of food products. Because food contains a variety of additives, further research should look into the rheological properties of LPSG at different pHs, as well as the presence of other salts and sugars often employed in the food industry. LPSG has the potential to be used in biomedical, pharmaceutical, food industries, tissue engineering, and cosmetic applications due to its biocompatibility, rheological properties, and antioxidant activities.

**Keyword:** Dynamic rheology, Intrinsic viscosity, Hydrocolloid, Salt

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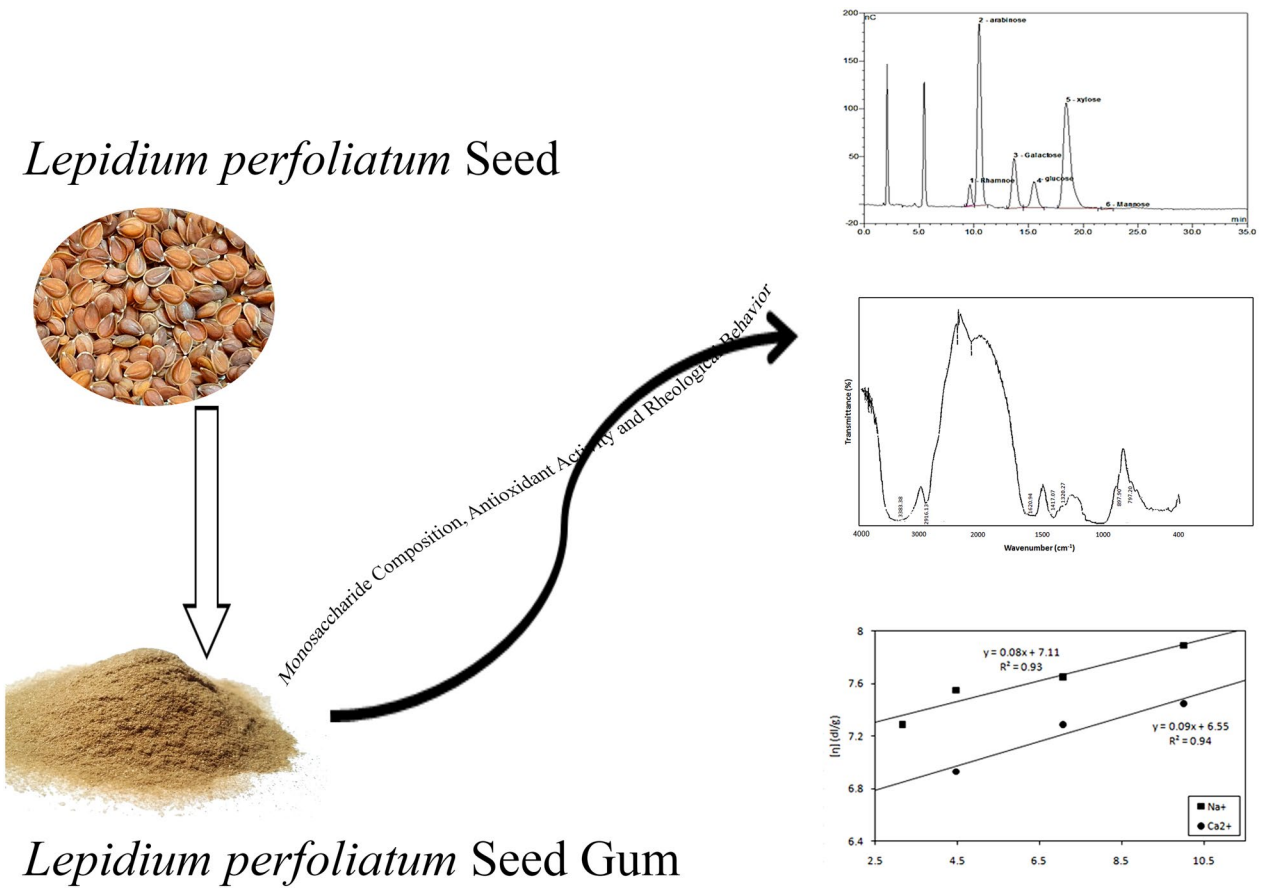
**Graphical Abstract**




## Background

Seed gums are plant-derived polysaccharides that are utilized in the food industry as thickening, stabilizing, gelling, and emulsifying agents [1, 2]. The rheological characteristics of hydrocolloids are extremely important because of the structural and textural properties of food products [3, 4].

*Lepidium perfoliatum* seed gum (LPSG) is a potential novel food thickening agent and an efficient food emulsion additive [5]. Recent studies demonstrated that LPSG can be applied as an alternative food hydrocolloid. LPSG has a lot of promise as a food thickener and stabilizer [6–8]. Steady shear flow behavior indicated that LPSG has high yield stress, consistency coefficient, and strong shear-thinning properties [6]. In our previous research, the dependence of rheological properties of LPSG on heating/cooling rate, temperature, and concentration was reported. The rheological behavior of LPSG indicated a weak gel behavior [9]. This gum can immobilize a large number of water molecules, increasing viscosity, stabilizing product consistency, and changing texture [10]. Due to the beneficial properties of LPSG, further study and use in the food industry should be considered.

Food formulations on an industrial scale typically include the inclusion of additives such as salt, which uses low molecular weight salts such as sodium chloride and calcium chloride to suppress microbial growth, improve sensory properties, and increase food shelf life [11]. The electrostatic force has a significant impact on the configuration of ionic polysaccharides and certain physicochemical characteristics such as viscosity. Since the cations affect the balance between repulsive and attractive forces between the molecules, there is a high gel strength or viscosity at specific concentrations of each cation and polymer [12]. Therefore, understanding the rheological behavior of hydrocolloids in the presence of salts is important. Several studies demonstrated that the effects of salt on the rheological properties of hydrocolloids varied depending on the salt types and their

concentration [12–16]. As a result, the influence of salts on hydrocolloid characteristics should be investigated under various conditions. The impacts of ion types and their concentrations on rheological behavior are important not only to specify whether the hydrocolloid behaves as a polyelectrolyte but also to determine functional rheological attributes. Because of the ionic nature of LPSG, additions of cations will affect the rheological properties of LPSG.

Despite the high potential of salts for hydrocolloid performance, no extensive studies on LPSG have been reported. Thus, the goals of this study were to study the rheological properties of LPSG in the presence of different concentrations of some common salts in foods (i.e., NaCl and $CaCl_2$) in dilute and concentrated regions. In addition, we investigated the monosaccharide composition, FTIR functional groups, and antioxidant properties of LPSG to assess its potential applications in food and medicine.

## Materials and methods

### Materials

*Lepidium perfoliatum* seeds were purchased in Mashhad, Iran, at a medicinal plant market. Authentication of the plant was performed at the Research Center for Plant Sciences of the Ferdowsi University of Mashhad under the supervision of the institute's expert plant taxonomist. The cleaned seeds were sealed in plastic bags and stored in a dry, cold place. Mucilage extraction was carried out using the procedure described by Koocheki et al. [5]. Briefly, *L. perfoliatum* seeds were soaked in distilled water at a water/seed ratio of 30:1 at 48 °C and pH of 8, for 1.5 h while stirring. The husks were extracted from the seed coats of *L. perfoliatum* seeds by mechanically milling the outer layer of seeds in a 27 cm basket centrifuge lined with a 1 mm mesh. The seeds were separated, and the dispersion was milled and sieved through a mesh 18 sifter after being dried overnight at 45 °C in a conventional oven. All chemicals used were of analytical grade.

### Monosaccharide composition analysis

According to Wood et al. the monosaccharide composition of LPSG was estimated using HPAEC-PAD [17]. After hydrolyzing LPSG in $H_2SO_4$ (1 N, 2 h, 100 °C), samples were diluted, filtered, and injected onto a CarboPac PA1 column (4 mm × 250 mm; Dionex, Sunnyvale, CA). Detection was conducted with a gold electrode using a pulsed amperometry detector. The monosaccharides were isolated as the post-column eluent by gradient elution from 100 mM NaOH to 300 mM NaOH with 600 mM NaOH. Monosaccharide standards included glucose, galactose, rhamnose, xylose, mannose, and arabinose. The instrument was operated and Dionex AI 450 software was used to process the data. Using galacturonic acid as a standard, the meta-hydroxydiphenyl approach was used to measure the content of uronic acid [18].

### FTIR

FTIR spectrometer Paragon 1000 (Perkin Elmer, Akron, OH, USA) was used in the measurements. FTIR spectra were recorded within a range of 4000–400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

### Dilute solution attributes

#### *Intrinsic viscosity measurement*

The volume of solvents (de-ionized water) applied to the stock solution (0.05 g/dL) was varied to produce dilute LPSG solutions. The viscosity of LPSG solutions in the presence of NaCl and $CaCl_2$ (0, 10, 20, 50, 100, and 200 mM) was determined using a size 75 Ubbelohde capillary viscometer (Cannon Instruments Co., Germany; $K = 0.01875\ mm^2s^{-2}$) immersed in a thermostatic water bath (Fan-Azma-Gostar, Tehran, Iran). All measurements were taken at least three times, with the average results recorded. The following formulas were used to compute the relative viscosity ($\eta_{rel}$) and specific viscosity ($\eta_{sp}$):

$$\eta_{\mathrm{rel}} = \frac{\eta}{\eta_0} \tag{1}$$

$$\eta_{\mathrm{sp}} = \frac{\eta - \eta_0}{\eta_0} = \eta_{\mathrm{rel}} - 1 \tag{2}$$

where $\eta$ is the dynamic viscosity of LPSG solution and $\eta_0$ is the de-ionized water's dynamic viscosity.

The $[\eta]$ was determined experimentally by measuring viscosity at quite low concentrations. Using the Huggins (Eq. 3) and Kraemer (Eq. 4) models, measurements were taken at various concentrations and extrapolated to infinite dilution [19, 20]:

$$\frac{\eta_{\mathrm{sp}}}{C} = [\eta] + k_H[\eta]C \tag{3}$$

$$\frac{\ln \eta_{\mathrm{rel}}}{C} = [\eta] + k_K[\eta]^2C \tag{4}$$

The Huggins constant, the Kraemer constant, and the concentration are all denoted by the letters $k_H$, $k_K$, and $C$, respectively.

The slope of $\eta_{rel}$ or $\eta_{sp}$ vs. concentration can also be used to measure $[\eta]$. Equations 5, 6, 7, and 8 have been established to calculate the $[\eta]$:

Tanglertpaibul and Rao [21]:

$$\eta_{\mathrm{rel}} = 1 + [\eta]C \tag{5}$$

Higiro et al. [22]:

$$\eta_{rel} = e^{[\eta]C} \tag{6}$$

$$\eta_{rel} = \frac{1}{1-[\eta]C} \tag{7}$$

Fedors [23]:

$$\frac{1}{2\left(\eta_{rel}^{\frac{1}{2}}\right)-1} = \frac{1}{[\eta]C} - \frac{1}{[\eta]C_{Max}} \tag{8}$$

where $C$ denotes the polymer concentration (g/dL) and $C_{max}$ denotes a factor showing the Fedors concentration limit.

### *Molecular conformation*

The $b$ part of Eq. 9 could be used to calculate the biopolymer conformation, which is the slope of a double logarithmic plot of $\eta_{sp}$ vs. concentration [24]:

$$\eta_{sp} = aC^b \tag{9}$$

### *Determination of chain stiffness parameter*

Using Eq. 10, salt tolerance ($S$) was calculated by plotting the slope of $[\eta]$ at various ionic strengths vs. the $I^{-0.5}$ (inverse square root of ionic strength) [15, 25]:

$$[\eta] = [\eta]_\infty + SI^{-0.5} \tag{10}$$

The $[\eta]$ at infinity ionic strength is denoted by $[\eta]$. Chain stiffness can be calculated using the $S$ parameter.

### Dynamic rheological measurements

Aqueous dispersions of LPSG were prepared at a concentration of 1% w/v in de-ionized water. Various concentrations of NaCl and $CaCl_2$ (0, 10, 20, 50, 100 and 200 mM) were added separately while stirring for 30 min. At the end, 10 mL of dispersions were shaken for 24 h at ambient temperature before being stored at 4 °C overnight for use in oscillation experiments. Small amplitude oscillatory shear was assessed using a Physica MCR301 controlled stress/strain rheometer (Anton Paar GmbH, Graz, Austria). For the measurements, a parallel plate device ($\phi$: 50 mm, gap: 1.000 mm) was used. Excess material was rubbed off with a spatula after each sample was transferred to the rheometer plate at room temperature. The samples were allowed to rest for 1 min at their initial temperatures before measurements. The data were evaluated using Rheoplus/32, version V3.40 (Anton Paar, MCR 301 Physica, VA, USA). At least one duplicate of each measurement was made.

The LVR must be defined before detailed dynamic measurements can be taken to analyze the sample's microstructure. The LVR for LPSG samples was calculated by taking amplitude sweep measurements

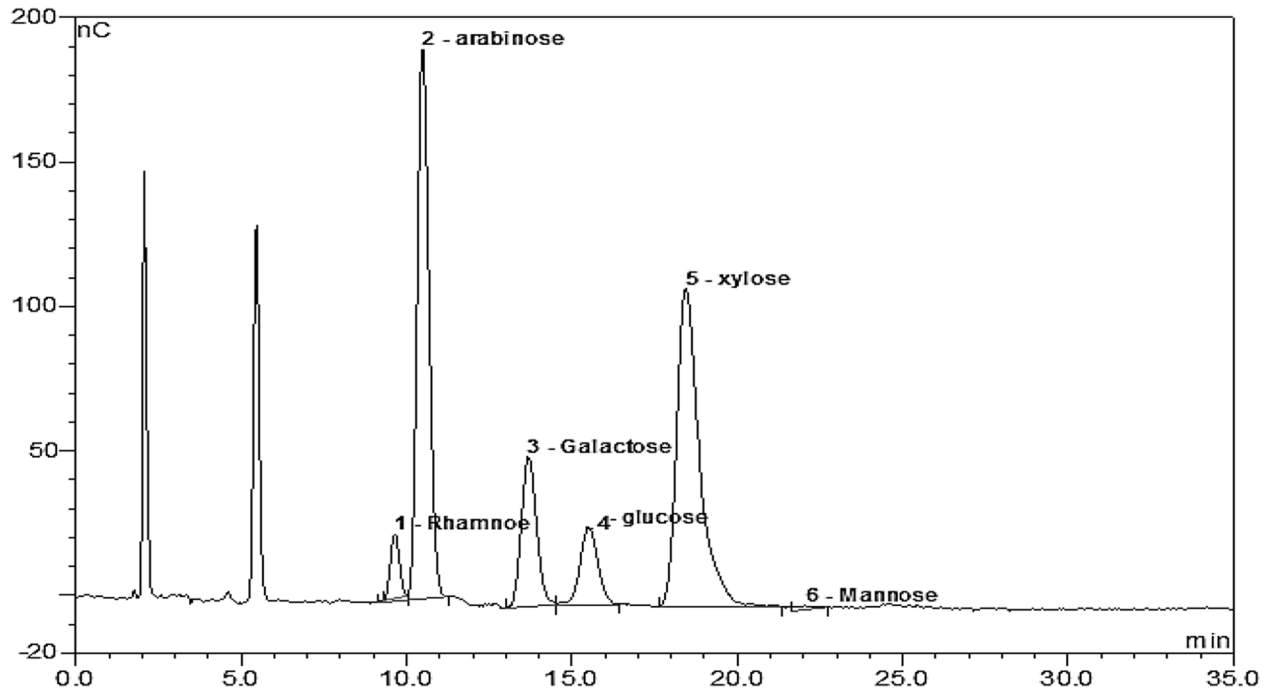



**Fig. 1** High-performance anion-exchange chromatograph of LPSG

(0.01–100%) at a constant frequency (1 Hz) and temperature (25 °C).

To assess the viscoelastic nature of LPSG in the presence of salts, frequency sweep tests at a constant strain in the LVR were performed. A strain of 0.5% was used in this test to disturb network formation as little as possible. The mechanical spectra were described by the storage ($G'$) and loss ($G''$) modulus values as a function of frequency in the 0.1–100 Hz range. The $G'$ is a measure of the sample's elastic component, and the $G''$ is a measure of the sample's viscous component.

### Antioxidant activities

#### *DPPH radical scavenging activity (DPPH-RSA)*

A modified procedure described by Ma et al. was used to evaluate the radical scavenging activity of DPPH [26]. In brief, 2 mL of LPSG dispersions (0.5–5 mg/mL) were mixed with 2 mL of freshly prepared DPPH in ethanol (0.15 mol/L). The mixture was thoroughly shaken before being set aside for 30 min at room temperature in the dark. The absorbance was determined at 517 nm. The following formula was used to estimate the DPPH radical scavenging activity:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \left(A_X - A_{X_0}/A_X\right)\right] \times 100 \quad (11)$$

#### *Ferric ion reducing antioxidant power (FRAP)*

The FRAP assay was determined using the method defined by Ma et al. [26]. In brief, 1.0 mL of LPSG dispersion (0.5–5 mg/mL) was mixed with 1.0 mL of phosphate buffer solution (0.2 M; pH = 6.6) and 1.0 mL of 1% (*w*/*v*) potassium ferricyanide solution and incubated at 50 °C for 20 min.

### Statistical analysis

For all statistical analyses, SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA). was used. One-way ANOVA and Duncan's multiple range test were performed to acknowledge any significant difference among rheological parameters. Differences in means were found statistically meaningful at the 95% ($p < 0.05$) confidence standard.

## Results and discussion

### Sugar compositions

Polysaccharide extracted from LPSG was mainly made up of rhamnose (3.40 ± 0.21), galactose (12.77 ± 0.01), arabinose (31.99 ± 0.48), glucose (7.15 ± 0.33), and xylose (44.66 ± 0.37), which differed from most other mucilage of seeds and indicates that it is most likely an arabinoxylan-type polysaccharide (Fig. 1). LPSG includes trace quantities of cellulose because *Lepidium perfoliatum*

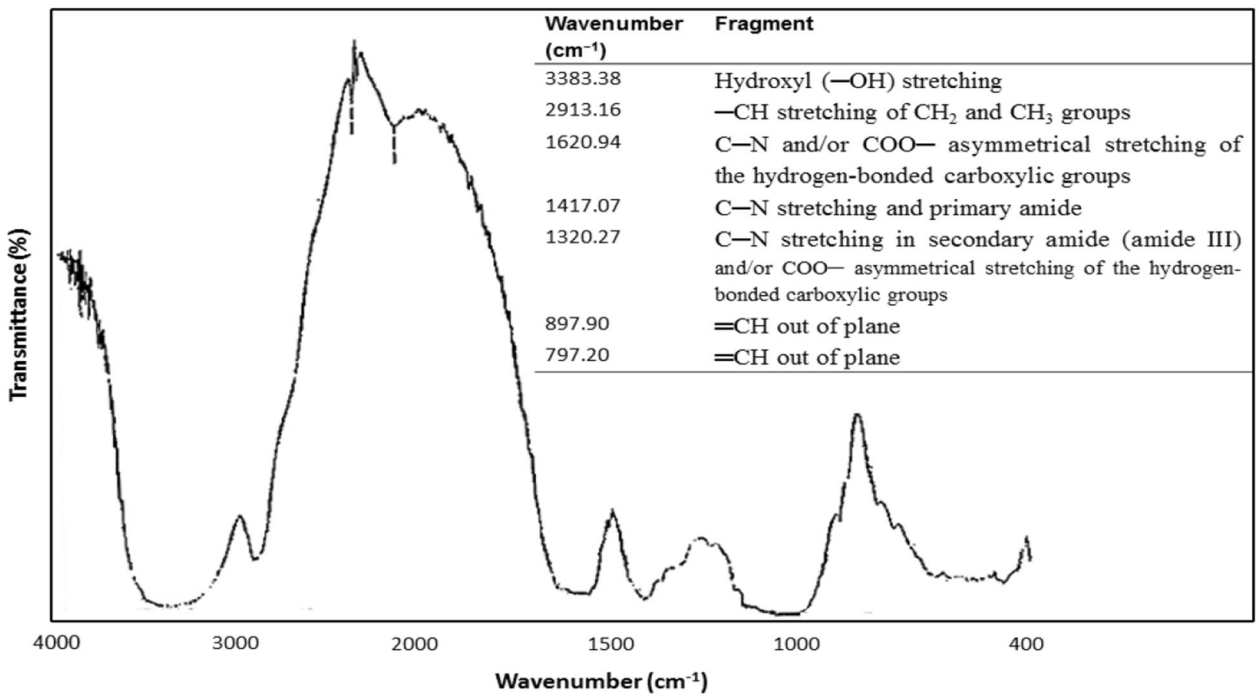



**Fig. 2** FTIR spectra of LPSG

**Table 1** Intrinsic viscosity values determined by six models for LPSG solutions at various concentrations of NaCl and $CaCl_2$

| Cosolute | Salt Conc. (mM) | Huggins (Eq. 3) | | Kraemer (Eq. 4) | | Tang. and Rao (Eq. 5) | | Higiro 1 (Eq. 6) | | Higiro 2 (Eq. 7) | | Fedors (Eq. 8) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $[\eta]$ | $R^2$ | $[\eta]$ | $R^2$ | $[\eta]$ | $R^2$ | $[\eta]$ | $R^2$ | $[\eta]$ | $R^2$ | $[\eta]$ | $R^2$ |
| Water | 0 | 5.44 ± 0.13 | 0.96 | 6.85 ± 0.08 | 0.77 | 15.73 ± 0.19 | 0.97 | 9.29 ± 0.13$^{a}$ | 0.99 | 7.39 ± 0.15 | 0.93 | 7.27 ± 0.18 | 0.99 |
| | 10 | 4.91 ± 0.07 | 0.91 | 5.79 ± 0.11 | 0.73 | 13.16 ± 0.17 | 0.97 | 7.89 ± 0.07$^{b}$ | 0.99 | 7.10 ± 0.17 | 0.96 | 6.91 ± 0.16 | 0.89 |
| | 20 | 4.69 ± 0.09 | 0.89 | 5.52 ± 0.10 | 0.74 | 12.91 ± 0.13 | 0.97 | 7.65 ± 0.11$^{bc}$ | 0.99 | 6.97 ± 0.11 | 0.90 | 6.72 ± 0.10 | 0.82 |
| NaCl | 50 | 4.19 ± 0.10 | 0.83 | 4.39 ± 0.09 | 0.69 | 12.30 ± 0.14 | 0.98 | 7.55 ± 0.08$^{cd}$ | 0.98 | 6.84 ± 0.08 | 0.89 | 6.41 ± 0.14 | 0.92 |
| | 100 | 3.49 ± 0.11 | 0.78 | 3.88 ± 0.07 | 0.78 | 11.88 ± 0.10 | 0.96 | 7.29 ± 0.11$^{d}$ | 0.98 | 6.76 ± 0.10 | 0.91 | 6.08 ± 0.11 | 0.88 |
| | 200 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 10 | 4.85 ± 0.11 | 0.87 | 5.01 ± 0.16 | 0.79 | 13.65 ± 0.10 | 0.96 | 7.45 ± 0.12$^{cd}$ | 0.99 | 7.14 ± 0.14 | 0.94 | 6.73 ± 0.16 | 0.90 |
| | 20 | 4.60 ± 0.09 | 0.71 | 4.47 ± 0.10 | 0.83 | 13.23 ± 0.09 | 0.98 | 7.29 ± 0.10$^{d}$ | 0.99 | 6.76 ± 0.13 | 0.96 | 6.59 ± 0.10 | 0.89 |
| $CaCl_2$ | 50 | 4.16 ± 0.14 | 0.87 | 4.23 ± 0.13 | 0.91 | 12.98 ± 0.03 | 0.98 | 6.93 ± 0.06$^{e}$ | 0.99 | 6.61 ± 0.10 | 0.89 | 6.24 ± 0.09 | 0.93 |
| | 100 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 200 | – | – | – | – | – | – | – | – | – | – | – | – |

Means labeled with the same letter are not significantly different ($P < 0.05$)

seeds are scraped and LPSG extracted from the seed wall. As a result, the cellulose cell wall, starch, and storage carbohydrate of the seed may provide part of the glucose found in gum. Plant cell walls are mostly made up of arabinoxylans [27, 28] and are very important for human nutrition as readily fermentable substrates for gut microbiota [29]. LPSG has a similar composition to flaxseed mucilage, which is made up of a mixture of neutral arabinoxylans and highly acidic rhamnose-containing polymers [30] and to that of *Plantago ovata* seed mucilage, which is also an arabinoxylan polysaccharide [31]. Aspinall stated that the cress seed mucilage also contains a xylopyranoarabinofuranan in addition to cellulose [32].

LPSG has 6.65% uronic acids in its structure, indicating its polyelectrolyte nature with a relatively low value of acidic polysaccharides. This value was higher than *Alyssum homolocarpum* seed gum [4] and was lower than those reported for seed gums such as sage seed gum (SSG) (28.2–32.2%), flaxseed gum (21.0–25.1%), psyllium gum (15.9%), Krasch seed gum (15.8%), and cress seed gum (15%) [33–37].

## FTIR

The FTIR technique was used to investigate the chemical structure of LPSG (Fig. 2). The stretching modes of C–H bonds of methyl groups ($-CH_3$) were aligned with the 2800–3000 $cm^{-1}$ wavenumber range, and the broadband at 3383 $cm^{-1}$ was derived from the presence of OH groups (hydroxyl stretching vibration of the polysaccharide). The free hydroxyl groups stretching bonds that actually occur in samples in the vapor phase as well as the bound O–H bands of carboxylic acid are two aspects that are indicated by the O–H stretching vibrations [37]. The C–H stretching vibration band was responsible for the peak at 2913.16 $cm^{-1}$ [38]; these include the stretching and bending vibrations of CH, $CH_2$ and $CH_3$, both symmetric and asymmetric, as well as occasional doubles overlapping with O–H [37]. The asymmetrical single bond –COO stretching vibration is responsible for the peak at 1620 $cm^{-1}$, whereas the symmetrical single bond –COO stretching vibration is responsible for the band at 1417 $cm^{-1}$. These –COO anti-symmetric and symmetric stretching bands of carboxylate may be related to uronic acids [4, 37]. As a result, the LPS *G′* s FT-IR spectra revealed the existence of carboxyl groups, which could act as ion-binding sites. These bands have a significant impact on the capacity to gel properly by interacting with water. Similar spectra were also observed for some food hydrocolloids such as guar gum and basil seed gum, which can bind with ions and form a gel due to the presence of these functional groups [40, 41].

## Dilute solution properties

Since the biopolymer chains separate in dilute solutions, the $[\eta]$ is proportional to the size and conformation of the macromolecular chains in a given solvent [42, 43]. Therefore, $[\eta]$ determination gives detailed insights into the molecular characteristics of a biopolymer [44]. The $[\eta]$ of LPSG solutions at different NaCl and $CaCl_2$ concentrations were calculated using six models (Table 1). The Higiro 1 equation, which calculates the $[\eta]$ via measuring the slope of natural logarithm of $\eta_{rel}$ vs. concentration plot, had a higher coefficient of determination than other equations which calculate the $[\eta]$ through measuring the intercept of plots. A similar result was observed by Sherahi for *Descurainia sophia* seed gum in the presence of NaCl and $CaCl_2$ [45].

**Table 2** Some molecular parameters of LPSG solutions in the presence of NaCl and $CaCl_2$

| Solvent | Water | NaCl | | | | | $CaCl_2$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 20 | 50 | 100 | 200 | 10 | 20 | 50 | 100 | 200 |
| *b* value | 0.96 | 1.10 | 1.19 | 1.28 | 1.31 | – | 1.14 | 1.21 | 1.33 | – | – |
| Berry number | 0.27–1.10 | 0.31–0.94 | 0.22–0.91 | 0.41–0.89 | 0.28–0.80 | – | 0.33–0.99 | 0.40–1.04 | 0.41–0.99 | – | – |
| Master curve slope | 0.68 | 0.70 | 0.73 | 0.86 | 0.92 | – | 0.96 | 1.07 | 1.09 | – | – |

The [$\eta$] of LPSG was observed to be 9.29 dL/g in deionized water (25 °C). This value was lower than those reported for *Plantago major* seed mucilage (14.24 dL/g) [2], *Alyssum homolocarpum* seed gum (18.33 dL/g) [4], Balangu seed gum (BSG) (72.3 dL/g) [46], SSG (24.32 dL/g) [47], and Tragacanthin (19.60 dL/g) [48]. However, the [$\eta$] of LPSG was greater than the values of basil seed gum (8.38 dL/g) [49], and *Mucuna flagellipes* seed gum (7.90 dL/g) [50]. Considering that the [$\eta$] is related to the molecular properties including molecular shape, molecular weight, chain conformation, and voluminosity of polysaccharide molecules [51], the difference in hydrocolloid structure and the rheological behavior is entirely different from one hydrocolloid solution to another [4].

The intrinsic viscosities appeared to have a decreasing trend as the ionic strength increased (Table 1). In the case of sodium ions, an increase in the ionic strength from 0 to 100 mM caused an abrupt drop in the [$\eta$] of LPSG from 9.29 ± 0.18 to 7.29 ± 0.27 dL/g. Increasing the ionic strength of LPSG solutions containing calcium ions to 50 mM, decreased the [$\eta$] of LPSG to 6.93 ± 0.38 dL/g (Table 1). These findings show that divalent salts had more ability to reduce the [$\eta$] of LPSG solution than monovalent salts [6, 15]. However, increasing the ionic strength to 100 mM for $Ca^{2+}$ and 200 mM for $Na^{+}$ caused the LPSG solution to precipitate completely. This decline may be attributed to the shielding effect of charges on the macromolecular chains [52]. Probably, this effect would become predominant by increasing the ionic strength of the solution; therefore, the aggregation between molecular species and a diminution in [$\eta$] would be observed.

When the concentration of the monovalent and divalent cations was increased, similar findings were obtained for SSG [53], BSG [46], *Descurainia sophia* seed gum [45], *Prunus armeniaca* gum [13], κ-carrageenan [54], and locust bean [42]. The impact of calcium ions on [$\eta$] was more considerable than that induced by sodium ions, meaning that monovalent salt was less efficient in reducing molecular dimensions than divalent salt. This is presumably because of the molecular cross-linking between LPSG and $Ca^{2+}$ and the occurrence of some aggregation,

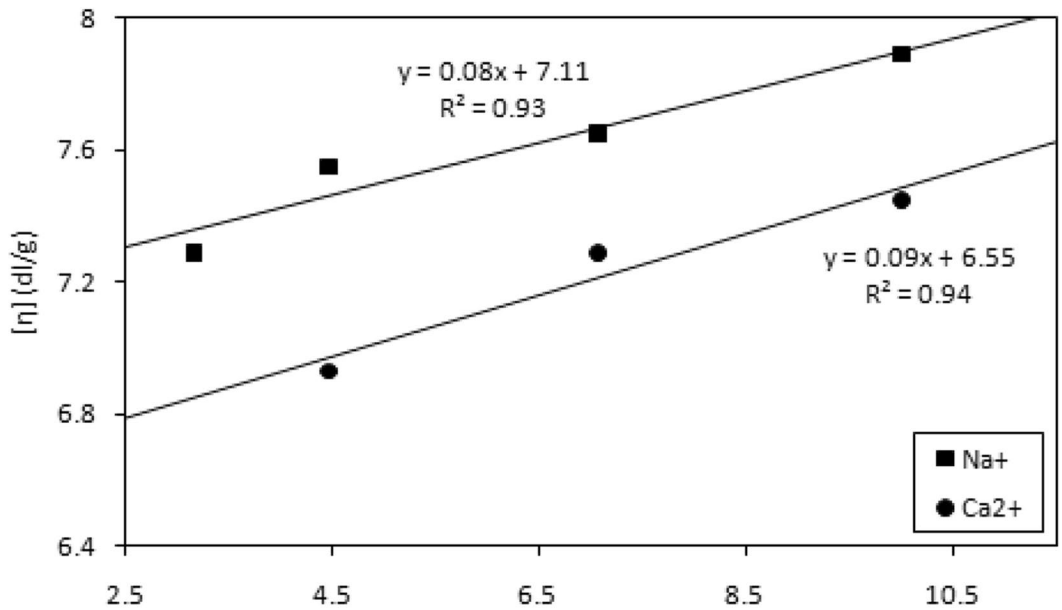



**Fig. 3** Dependence of [$\eta$] on $I^{-0.5}$ for LPSG solutions. Values represent the means of measurements in three replicates

which resulted in a higher amount of molecular contraction [55].

#### *Molecular conformation*

The most predominant polysaccharide conformation in the dilute domain is the random coil structure in which the molecules oscillate sequentially via Brownian motion [56]. The single polysaccharide coils move freely in a dilute solution, because they are far enough apart and have little effect on each other [57]. A double logarithmic plot of specific viscosity vs. concentration is used to evaluate the coil overlap factor and the dilute Newtonian domain [58].

The slopes of the master curves in the dilute domain and concentrated regime are usually about 1.4 and 3.3, respectively [58]. The power equation slope ($b$), berry number ($C[\eta]$), and master curve's slope of LPSG solutions at tested conditions were shown (Table 2). The slopes of master curves were in the range of 0.68–0.92, and 0.68–1.09 when NaCl and $CaCl_2$ were added to LPSG solutions, respectively. Hence, it can be observed that all LPSG solutions were in the dilute region without molecular entanglements and coil overlapping at all cosolute concentrations. Furthermore, the Berry number was within the range of 0.22–1.10 at all tested conditions, displaying once again that no molecular entanglements and coil overlaps occurred (Table 2). When the Berry number exceeds unity, the molecular entanglement and coil-overlapping start to occur in the concentrated domain [4].

Some researchers have stated that in the dilute domain, the slope of the power-law model ($b$ value) greater than unity is associated with an entanglement [58] or random coil conformation [56], while the lower ones are associated with rod-like conformation [4]. The $b$ values of LPSG solutions changed from 1.10 to 1.31 and from 1.14 to 1.33 in the presence of NaCl and $CaCl_2$, respectively (Table 2). It represented that the molecular conformation of LPSG is probably a random coil in the presence of these cations [37, 59]. This may be due to the shielding effect of charges on polyelectrolyte chains [45]. Moreover, the $b$ value increased when ions concentration increased, which expressed that they were able to promote the random coil conformation in LPSG solutions. Hence, the molecular conformation of LPSG was still a random coil in the presence of these cation salts. Similar results were obtained for cress seed gum [59], BSG [46] and *Descurainia sophia* seed gum [45]. In comparison to these findings, Lai and Chiang indicated that the $b$ value for hsian-tsao leaf gum varied from 0.78 to 0.8 in the dilute regime, concluding that the molecular conformation was more rod-like than random coil [60].

#### *Estimation of the chain stiffness parameters*

Based on Eq. 10, a plot of $[\eta]$ vs. $I^{-0.5}$ was outlined to determine the stiffness parameter ($S$) and $[\eta]$ for LPSG solutions at infinite ionic strength ($[\eta]_\infty$) of $Ca^{2+}$ and $Na^+$ ions (Fig. 3). It is clear that there was a linear trend for both ions studied ($R^2 > 0.93$), consistent with the relationship described by Smidsrød and Haug [25]. In this regard, $[\eta]_\infty$ was found to be 7.11 dL/g and 6.55 dL/g for LPSG solutions in the presence of sodium and calcium ions, respectively.

The chain stiffness values for LPSG in NaCl and $CaCl_2$ solutions were 0.08 and 0.09, respectively. The value of chain stiffness factor for LPSG in the presence of $Na^+$ was lower than that reported for tragacanthin (0.6) [48], BSG (0.346) [46], and SSG (0.381) [53]. In addition, in the $CaCl_2$ solution, this parameter was lower than that for BSG (0.507) [46] and SSG (0.821) [53], which shows that LPSG had a rather flexible conformation. The higher values of the stiffness parameter for divalent ions indicate that more interactions in LPSG chains were made.

**Table 3** Strain sweep parameters of LPSG solutions in the presence of NaCl and $CaCl_2$ (strain %: 0.5; frequency: 1 Hz; 25 °C)

| Cosolute | Salt Conc. (mM) | $G'_{LVR}$ (Pa) | $G''_{LVR}$ (Pa) | $\gamma_L$ (%) | Tan $\delta_{LVR}$ |
|---|---|---|---|---|---|
| NaCl | 20 | $22.9 \pm 0.6^{b}$ | $6.43 \pm 0.5^{ab}$ | 1.46 | 0.28 |
| | 50 | $20.1 \pm 0.2^{a}$ | $5.97 \pm 0.2^{a}$ | 4.60 | 0.29 |
| | 100 | $20.6 \pm 0.4^{a}$ | $6.10 \pm 0.1^{a}$ | 4.61 | 0.28 |
| | 200 | $27.6 \pm 0.6^{c}$ | $7.15 \pm 0.3^{b}$ | 1.45 | 0.25 |
| $CaCl_2$ | 20 | $46.1 \pm 0.4^{d}$ | $10.1 \pm 0.1^{c}$ | 0.99 | 0.22 |
| | 50 | $23.9 \pm 0.3^{b}$ | $6.0 \pm 0.2^{a}$ | 0.99 | 0.25 |
| | 100 | $23.6 \pm 0.2^{b}$ | $5.9 \pm 0.2^{a}$ | 1.46 | 0.25 |
| | 200 | $21.4 \pm 0.2^{a}$ | $5.6 \pm 0.2^{a}$ | 2.13 | 0.26 |

Means labeled with the same letter are not significantly different ($P < 0.05$)

### Dynamic rheological measurements

#### *Strain sweep measurements*

The experimental results regarding the effect of ionic strength on the limiting values of storage and loss moduli, strain ($\gamma_L$), and tan $\delta_{LVR}$ of LPSG solutions are summarized in Table 3. The strain at which storage modulus decreased sharply is defined as the critical strain. The critical strain is reflected to the maximum deformability that the hydrocolloid could sustain without structural collapse [61]. In addition, most solid foods have LVR in the range of 0.1–2% [62].

The $G'$ of LPSG solution remained constant at the strain of about 1% at low $CaCl_2$ concentrations. With increasing divalent cation concentration, the $\gamma_L$ increased to more than 2% (Table 3). This shows that increasing

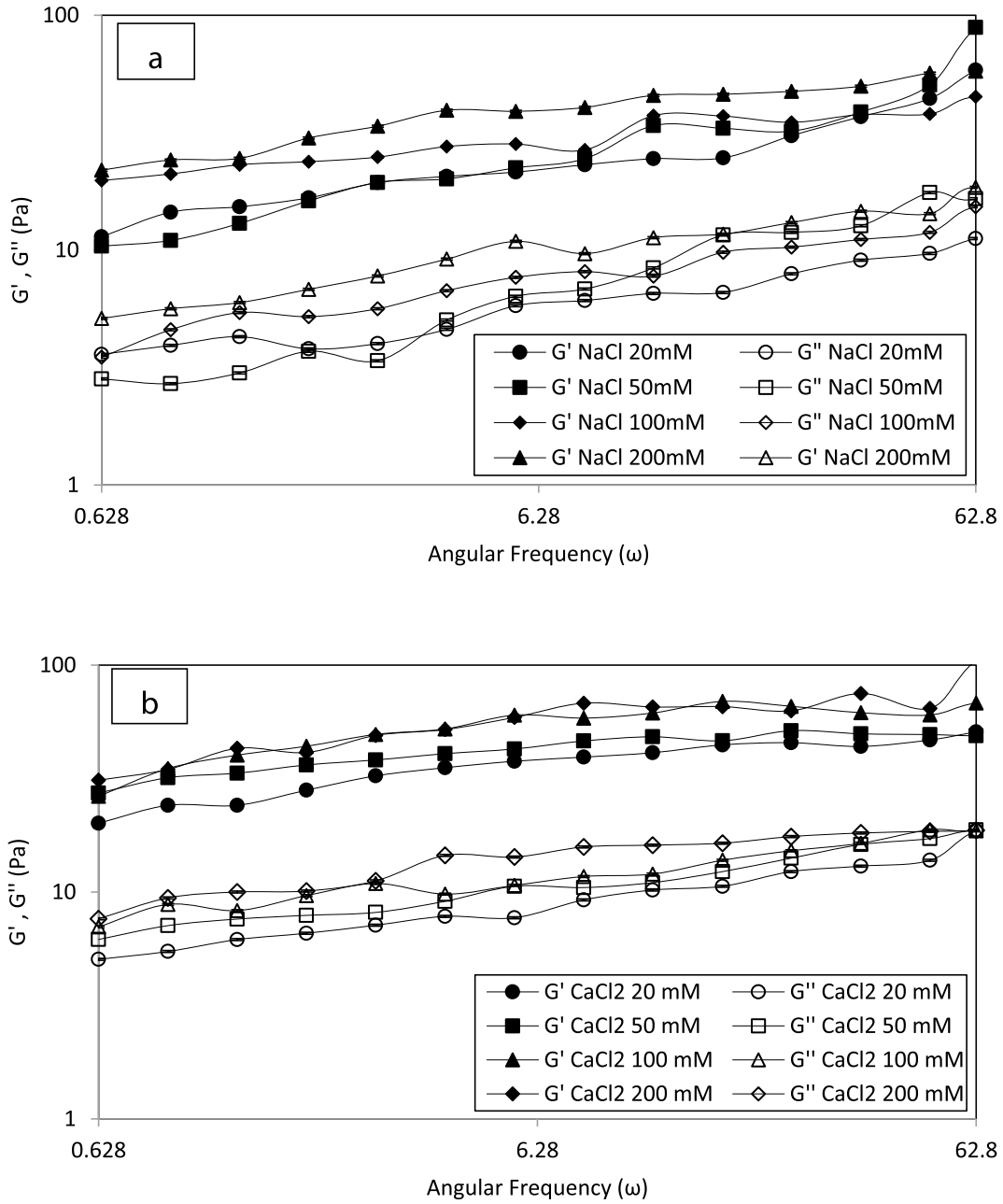



**Fig. 4** Frequency sweep profile at 0.5% strain for the LPSG solutions in the presence of different NaCl (**a**) $CaCl_2$ (**b**) concentrations at 25 °C (closed symbols, *G'*; open symbols, *G''*). Values represent the means of measurements in three replicates

the $CaCl_2$ concentration increased the resistance to elastic deformation (yield strain) in the LPSG solution. This may be due to the interchain interactions and therefore an increase in the stiffness of mucilage solutions in the concentrated domain in the presence of divalent ions [6].

The $\gamma_L$ was at its highest level in the presence of 100 mM NaCl. However, at higher concentrations of monovalent salt (200 mM), the $\gamma_L$ decreased. This decrease might be attributed to the progressive suppression of intermolecular charge-charge repulsion and consequent contraction of the polysaccharide molecules [14]. A similar reduction was reported by Sherahi et al. for *Descurainia sophia* seed gum [45]. It has been reported that the affinity of LPSG for cations is proportional to the charge/ion radius ratios and small ions with high charge have a stronger affinity for chain binding sites [6, 63, 64].

**Table 4** The viscoelastic parameters of LPSG solutions in the presence of different concentrations of NaCl and $CaCl_2$ as quantified by frequency sweep tests (frequency: 1 Hz; strain %: 0.5; 25 °C)

| Cosolute | Salt Conc. (mM) | $G'$ (Pa) | $G''$ (Pa) | Tan $\delta$ |
|---|---|---|---|---|
| NaCl | 20 | $21.5 \pm 1.0^{a}$ | $5.7 \pm 0.8^{a}$ | 0.26 |
| | 50 | $22.4 \pm 0.9^{a}$ | $6.4 \pm 0.9^{ab}$ | 0.28 |
| | 100 | $28.3 \pm 0.6^{b}$ | $7.7 \pm 0.7^{b}$ | 0.27 |
| | 200 | $39.0 \pm 1.4^{c}$ | $10.9 \pm 0.1^{c}$ | 0.28 |
| $CaCl_2$ | 20 | $37.7 \pm 1.8^{c}$ | $7.7 \pm 0.5^{b}$ | 0.21 |
| | 50 | $42.7 \pm 1.1^{d}$ | $10.6 \pm 0.2^{c}$ | 0.25 |
| | 100 | $60.2 \pm 1.9^{e}$ | $10.7 \pm 0.6^{c}$ | 0.18 |
| | 200 | $58.8 \pm 2.2^{e}$ | $14.3 \pm 0.9^{d}$ | 0.24 |

Means labeled with the same letter are not significantly different ($P < 0.05$)

An increase in the divalent salt concentration reduced viscoelastic modulus, as expected for such polyelectrolytes. According to Medina-Torres et al., since the hydrocolloid is a negatively charged polyelectrolyte molecule, the addition of positive ions decreases the repulsion forces and causes the molecules to expand, resulting in a decrease in the viscoelastic modulus [65]. The $G'$ and $G''$ reductions were more dependent on the $Ca^{2+}$ ion rather than $Na^{+}$ concentration. These results suggest that LPSG is a negatively charged polyelectrolyte molecule. The values of storage and loss modulus decreased with the addition of divalent salt, indicating that the structural strength ($G'_{LVR}$) of the system was reduced. Turkoz et al. [66] and Rezagholi et al. [67] found a similar phenomenon for quince seed mucilage and xanthan gum, respectively.

The tan $\delta$ is a beneficial parameter for determining a sample's viscoelasticity at a given frequency [68]. Tan $\delta$ values lower than 1 mean that the sample is predominantly elastic [3]. As shown in Table 3, the tan $\delta$ values for LPSG in the presence of mono and divalent salts were smaller than unity (0.22–0.29), indicating that the solutions were more elastic than viscous. Tan $\delta$ in the numerical range of 0.2–0.3 is reported for amorphous polymers [3].

#### *Frequency sweep measurements*

As LPSG behaves as a polyelectrolyte, the rheological properties are affected by the addition of salts. The value of the storage modulus was always higher than the loss modulus and no crossover point occurred, representing typical weak gel-like behavior (Fig. 4). Therefore, LPSG solutions behaved more like an elastic material, meaning that the deformation will mostly be elastic and recoverable. As shown in Table 4, the tan $\delta$ values of the samples in the presence of different concentrations of cations were lower than 1, indicating that the solutions were more elastic than viscous.

As shown in Fig. 4, the rheological characteristics of LPSG were influenced by the addition of mono- and divalent salts due to ionic nature of the biopolymer [6]. These parameters changed more severely with the addition of calcium ions rather than sodium ions. The less frequency dependency and greater increase in the elastic modulus with the addition of $CaCl_2$ demonstrate the occurrence of

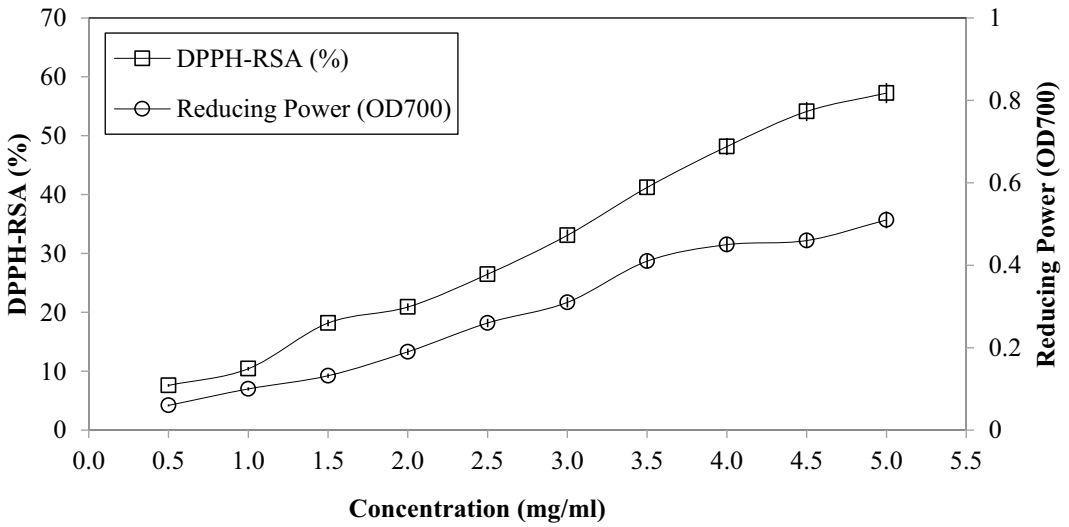



**Fig. 5** Evaluation of the antioxidant activity of LPSG: **A** DPPH-RSA; **B** Reducing power. Values represent the means of measurements in three replicates. Vertical bars represent standard errors

a change in the network structure of LPSG from concentrated solutions to elastic gels [60, 61].

The $G'$ and $G''$ of LPSG in the presence of $CaCl_2$ slightly increased with the addition of salts to 200 mM. The presence of ions may promote interactions between chains and thus an increase in the viscosity [64]. It seems that $Ca^{2+}$ could modify the network structure of LPSG through cross-linking with carboxyl groups. Similar observations have been reported by Lin et al. [12] for mulberry leaf hydrocolloids. Rodrıguez-Hernandez et al. stated that the stronger carboxylate–cation$^{2+}$–carboxylate interactions evolve a higher capacity of adjacent helices cross-linking when divalent ions are involved [14]. Similar results to these promoted interchain interactions and increased junction zones were reported for *E. contortisiliquum* gum [64].

$G'$, $G''$, and Tan $\delta$ at the frequency of 1 Hz for samples containing mono and divalent salts are tabulated in Table 4. The $G'$ and $G''$ steadily increased with the addition of mono and divalent salts. It is believed that $Na^+$ ions may create indirect cross-linking with the assistance of water [12]. By shielding the electrostatic repulsion of the carboxylate groups, polyanion-ion$^+$-water-ion$^+$-polyanion linkages between the adjacent chains of other linkages could be evolved [64, 69]. As mentioned before, the $G'$ of LPSG increased with the addition of 20–200 mM $CaCl_2$. In justifying this phenomenon, the researchers stated that the formation of stronger carboxylate–cation–carboxylate interactions render a higher capacity of adjacent helices cross-linking when divalent cations are involved [14]. As a result, an increase in the storage modulus may be observed in the presence of salts. Comparing the effect of NaCl and $CaCl_2$ on $G'$ of LPSG solutions showed that calcium ions had greater effects than sodium ions.

### Antioxidant activity analysis

The DPPH-RSA is focused on the transfer of hydrogen atoms. This assay has been frequently used to evaluate the antioxidant activities of food materials, among others [70]. A lower absorbance indicates a greater ability to scavenge DPPH-free radicals [71]. The DPPH-RSA of LPSG at concentrations ranging from 0.5 to 5.0 mg/mL is shown in Fig. 5. The DPPH-RSA of LPSG increased steadily, with the maximum DPPH-RSA at 5.0 mg/mL being roughly 57.24%. The LPSG concentration that scavenges 50% of free radicals (IC50) was determined to be 4300 µg/mL. The DPPH-RSA of LPSG was higher than Flixweed seeds mucilage [72] and Hsian-tsao Leaf gum [73], gum mastic [74], and *Plantago major* seed mucilage [2].

The $Fe^{2+}$ concentration in samples is used in the FRAP-assay to determine the existence of antioxidants and the potential reductive ability [75]. It was discovered that LPSG can convert $Fe^{3+}$ to $Fe^{2+}$ (Fig. 5). The reducing ability of LPSG rose in direct proportion to the concentration of the sample. The FRAP values of LPSG increased steadily from 0.06 to 0.51 mM/l by elevating the concentration from 0.5–5 mg/mL. The obtained findings were lower than those reported for fenugreek seed gum (0.25–0.60 mM/l) [76] and polysaccharides derived from Hawk tea (0.20–1.52 mM/l) [77], but similar to those reported for Chuanxiong rhizome (0.01–0.70 mM/l) [78] and Plantago major seed gum (0.12 to 0.37 mM/l) [79]. According to the findings, LPSG was found to be an effective radical scavenger.

## Conclusion

The effects of NaCl and $CaCl_2$ salts on the dilute solution and dynamic rheological properties of LPSG solutions were studied in this research to shed light on their behavior in real systems. The results showed that LPSG is an arabinoxylan-type polysaccharide. In addition, 44.66% xylose, 31.99% arabinose, 12.77% galactose, 7.15% glucose, and 3.40% rhamnose were found in HPAEC. Rheological measurements of LPSG in the dilute region in the presence of NaCl and $CaCl_2$ revealed that increasing the ion strength led to a decrease in the $[\eta]$. In other words, the intrinsic viscosities tend to decrease as ionic strength increases. The Higiro 1 equation was found to be the most accurate model for predicting the $[\eta]$ of LPSG solutions at different ion types and ionic strengths. The observed $b$ values for LPSG solutions under the evaluated conditions were in the 0.96–1.33 range, indicating that the molecular conformation of LPSG is a most likely random coil. Overall, it is possible to infer that adding salt, regardless of the type of salt, significantly reduced the solvent quality. At all ion concentrations, SAOS tests of LPSG solutions revealed a weak gel-like behavior. The tan $\delta$ values showed that LPSG can form weak elastic gels throughout the specified frequency range in the presence of both salts. The $G'$ and $G''$ of LPSG solutions in the presence of NaCl and $CaCl_2$ steadily increased with the inclusion of salts. Calcium ions had greater effects on the rheological parameters than sodium ions. According to the findings of this study, the type and concentration of salts affected the rheological properties of LPSG dispersions. Hence, trying to adjust the salt concentration could modify the rheological properties of food products. This is a significant challenge, particularly in the production of food compositions with suitable sensory, functional, and rheological features. Because food contains a variety of additives, further research should look into the rheological properties of LPSG at different pHs, as well as the presence of other salts and sugars often employed in the food industry. LPSG has the potential to be used in

biomedical, pharmaceutical, food industries, tissue engineering, and cosmetic applications due to its biocompatibility, rheological properties, and antioxidant activities.

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**Author contributions**
"Conceptualization, AK; methodology, AK and MAH; software, MAH; investigation, MAH; data curation, AK; writing—original draft preparation, MAH; writing—review and editing, AK and MRM; supervision, AK; funding acquisition, AK. All athors have read and agreed to the published version of the manuscript." All authors read and approved the final manuscript.

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## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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