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Characterizations and rheological study of the purified

polysaccharide extracted from quince seeds

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Abstract

Background

The functional characteristics of hydrocolloids are mainly dependent on their physicochemical properties. Thus, it is essential to characterize the new sources of hydrocolloids.

Results

Quince seed gum (QSG) is a high molecular weight polysaccharide (9.61×10^6 g/mol) composed of $85.04 \pm 2.87\%$ carbohydrate (6.39% L-arabinose, 40.43% D-xylose, 5.60% D-galactose, 5.75%

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D- glucose and 31.11% D-mannose), 13.16±1.73% uronic acid, 5.77±0.83% moisture, 2.78±0.21 % protein, 5.64±0.21% ash, and 0.75±0.09% fat. Our findings indicated that this gum could be introduced as a value-added by-product in the food and pharmaceutical industries. ¹³C NMR and FT-IR suggested a highly substituted xylan structure for QSG. In dilute regime, an increase in the ion concentration was accompanied by a decrease in intrinsic viscosity of QSG. When the salt concentration increased from 0 to 50 mM, the consistency coefficient (as a measure of apparent viscosity) declined. On the other hand, with further increasing of salt concentration, the consistency coefficient (as a measure of apparent viscosity) values increased. Similarly, G' and G'' values for 10 and 50 mM CaCl₂ concentrations were less than control samples.

Conclusion

The rheological behavior of the QSG studied in this paper can provide insight into its potential application in food and pharmaceutical industries.

Keywords: Chemical composition; Chemical structure; Dilute solution properties; Dynamic rheological behavior; Quince seed gum; Steady-state properties.

1. Introduction

The application of hydrocolloids in food and pharmaceutical systems is enormous, notably as thickener, stabilizer, edible coating and fat replacer ¹. Additionally, because of their low cost and extensive functional properties, hydrocolloids are widely utilized in different applications ².

Quince is a member of the Rosaceae family, which is a native fruit of west Asian region. This fruit is widely distributed in Caucasus region, Dagestan, Afghanistan, and Antalya ³. Quince seed mucilage is used in Iran to prevent asthma, relieve a cough and chest discomfort. Jouki, Mortazavi, Yazdi and Koocheki ⁴ indicated that the optimum extraction condition of quince seed

gum (QSG) that achieved outstanding properties with highest yield (11.58%), antioxidant activity (29.88%), viscosity at shear rate of 46.16 1/s (1473.96 mPa.s), emulsion stability (94.89%), foam stability (21.36%) and lowest turbidity (068 \dot{A}) and protein content (2.71%) were conducted with extraction temperature of 65 °C, for 5 min, and the water/seed (W/S) ratio of 25.1:1. Abbastabar, Azizi, Adnani and Abbasi ⁵ investigated the rheological behavior of QSG and found that the flow behavior of QSG was shear thinning. The dynamic rheological measurement showed that the viscoelastic range decreased with the addition of 0.2 NaCl solution, but elastic modulus showing an increasing trend. Dilute solution investigation demonstrated that QSG has high hydrodynamic volume, and as a result has high gelling ability in aqueous solution.

There are numerous investigations on the physicochemical and structural properties of food hydrocolloids ⁶. The functional characteristics of biopolymers are profoundly dependent on their structural and chemical properties such as chemical composition, conformation, the sequence of monosaccharide, configuration, and position of glycoside linkage⁶. Therefore, it is necessary to investigate the physicochemical and structural characteristics of new sources of hydrocolloids.

In the present research, the gum from quince seeds was extracted. After purification, dilute solution behavior, steady-state and dynamic rheological properties of this gum were evaluated. Furthermore, structural properties of QSG were analyzed by carbon-13 nuclear magnetic resonance (¹³C NMR), and Fourier transform infrared spectrophotometry (FT-IR) while its monosaccharides composition was analyzed using anion exchange chromatography (HPAEC). Gel permeation chromatography (GPC) was used to investigate molecular properties of QSG such as weight average molecular weight (Mw), number average molecular weight (Mn), and polydispersity index (PDI).

2. Materials and methods

2.1. Extraction and Purification of polysaccharide

Quince seed purchased from local markets in Mashhad, Iran. Quince seed gum (QSG) was extracted, at optimized conditions according to the method reported by Jouki, Mortazavi, Yazdi and Koocheki ⁴. To purify QSG, extracted gum was dissolved in deionized water (200 rpm for 60 min). The samples were then boiled in 80% ethanol to separate proteins with low molecular weight. The precipitated samples were dissolved in deionized water and stored at 4°C overnight to complete hydration. Any insoluble materials were removed by centrifugation at 5000 g for 10 min. H₂O₂ was added to the supernatant at 25 °C for 4h to oxidize and remove any colored compounds. Soluble substances were again precipitated with four volumes of ethanol, freeze-dried and then stored in a desiccator with silica gel for further experiments.

2.2. Analytical method

Purification yield (%) was determined as the dry weight of the purified gum relative to seed weight. In order to determine moisture, fat, ash and protein contents of QSG samples, recommended methods by AOAC were used ⁷. The protein content was quantified using a factor of 6.25 (nitrogen to protein conversion factor). In order to determine total carbohydrate content in QSG samples, the phenol-sulfuric acid method was used. The uronic acid measurement was performed using the m-hydroxydiphenyl method ⁸.

2.3. Monosaccharide composition

The monosaccharide analysis was performed by a well-established procedure recommended by the National Renewable Energy Laboratory (NREL) NREL with two-step acid hydrolysis and quantified using HPAEC-PAD. In brief, ca. 30 mg dry matter QSG material was mixed with 72% H₂SO₄ and left to react at 30 °C for 1 h. The reaction mixture was then diluted to 4% H₂SO₄ and hydrolyzed in an autoclave at 120 °C for 40 min. (Rhein-knudsen et al. 2017). HPAEC separation of the QSG was performed using an HPAEC-PAD, ICS5000 system (Dionex Corp. Sunnyvale, CA) equipped with a CarboPac™ PA1 column by a method principally as described by Arnous and Meyer ⁹. L-fucose, L-arabinose, L-rhamnose, D-galactose, D-glucose, D-xylose, D-mannose, D-galacturonic acid, and D-glucuronic acid were used as monosaccharide standards for quantification.

2.4. Elemental analysis

Quantification of elements was operated using an earlier explained method ¹⁰. 0.5 g gum sample was mixed with nitric acid and hydrogen peroxide (2:1) in perfluoroalkoxy (PFA) digestion vessel. Afterward, a microwave oven was used for decomposition of the sample (400 W for 15 min). Finally, the samples were cooled to 25 °C, and the volume made up of deionized water. Mineral profile of QSG was determined using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (SPECTRO ARCOS, Ametek. Germany).

2.5. Molecular parameter measurements

The values of M_n and M_w of QSG sample were measured by gel permeation chromatography (GPC) equipped with a PL Aquagel-OH Mixed-H column. A solution of QSG sample was prepared with deionized water and filtered through a 0.2 μm filter, followed by injection at a constant flow rate of 1 ml/min. The eluent water was used and monitored with refractive index detector ¹. For calibration, a standard curve was constructed using dextran molecular weight standards with a molecular weight between 5200 and 988,000 g/mol.

2.6. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of QSG sample was recorded on FT-IR spectroscopy (AVATAR 370 FT-IR, Thermo Nicolet). The spectrum was obtained in a range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} .

2.7. ¹³C nuclear magnetic spectroscopy (NMR)

5 mg of the polysaccharide sample was dissolved in D_2O with continuous stirring for 2 h. With an Avance DRX-500 Bruker Spectrometers, equipped with a process controller, ¹³C NMR spectra were recorded.

2.8. Intrinsic viscosity

A range of QSG solution was prepared from a stock solution (0.3 g dL^{-1}) to evaluate dilute solution properties. The measurements were carried out at 25 °C using a capillary viscometer (KPG Ubbelohde, $K = 0.00753$).

Kraemer, Huggins, Higiroy and Tanglerpatibul and Rao are most common models used to estimate intrinsic viscosity. Therefore, these models were used to facilitate comparison of the intrinsic viscosity obtained for QSG and other natural polymers.

Kraemer model ¹¹:

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k_k [\eta]^2 C \quad (1)$$

Huggins model ¹²:

$$\frac{\eta_{sp}}{C} = [\eta] + k_H [\eta]^2 C \quad (2)$$

where, k_H , k_K , and C are Huggins constant, Kraemer constant and solute concentrations, respectively.

Tanglertpaibul-Rao's equation ¹³:

$$\eta_{rel} = 1 + [\eta] C \quad (3)$$

Higiroy's models ¹⁴:

$$\eta_{rel} = e^{[\eta]C} \quad (4)$$

$$\eta_{rel} = \frac{1}{1 - [\eta]C} \quad (5)$$

2.8.1. Estimation of the molecular conformation

The slope of a double logarithmic plot of specific viscosity versus concentration is a measure of polysaccharides conformation ^{15, 16}. Eq. 6 was used to determine this parameter:

$$\eta_{sp} = aC^b \quad (6)$$

2.8.2. Salt tolerance (S) and relative stiffness parameter (B)

The intrinsic viscosity's slope at different ionic strengths versus the inverse square root of ionic strength ($I^{-0.5}$) was plotted to determine salt tolerance parameter (S) ¹⁷:

$$[\eta] = [\eta]_{\infty} + SI^{-0.5} \quad (7)$$

Where, $[\eta]_{\infty}$ is the intrinsic viscosity of infinite ionic strength. S parameter is a measure of chain stiffness.

Based on Smidsrød and Haug ¹⁸, S parameter is notably dependent on molecular weight of the polymer. Thus, they developed a semi-empirical assay (B value method) to overcome this problem. The relative stiffness parameter (B) was quantified from the intrinsic viscosity at an ionic strength of 0.1 according to the following formula:

$$S = B (\eta_{I=0.1})^v \quad (8)$$

In which, v is usually ranged between 1.2 to 1.4 for most polyelectrolyte ¹⁹. An average value of 1.3 was used in this paper.

2.9. Steady shear measurements

Steady shear evaluation of the gum solutions in the presence of CaCl₂ was carried out by a rotational viscometer (R/S plus Rheometer) equipped with a heating circulator at 25 °C using an SC4-18 spindle. All the measurements were performed over a wide range of shear rate from 1 to 300 s⁻¹.

Steady shear properties such as consistency coefficient and flow behavior index values were quantified using the power law equation:

$$\tau = k\dot{\gamma}^n \quad (9)$$

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

2.10. Small amplitude oscillatory shear measurements

Dynamic rheological measurements were conducted with a HAAKE MARS III rheometer (Thermo Scientific, Karlsruhe, Germany) equipped with a Peltier plate for temperature control. Each sample was transferred onto the rheometer plate using a “parallel plate sensor” PP35/1Ti (diameter of 35 mm) at the room temperature, and excess material was wiped off with a spatula. For equilibration, all samples were allowed to rest at the initial temperatures for 1 min. The RheoWin software 3.61 (Thermo Fisher Scientific) was employed for data evaluation.

The linear viscoelastic region (LVR) was analyzed for detailed dynamic measurements to evaluate the sample's microstructure. The linear viscoelastic region (LVR) for QSG samples was determined by performing an amplitude sweep measurements (0.01–100%) at a constant frequency (1 Hz) and temperature of 25 °C.

Frequency sweep tests at a constant strain in the LVR region were carried out to determine the viscoelastic nature of QSG (0.5%). In this test, a strain of 0.5 was applied in order to disturb as less as possible the network formation. The mechanical spectra were characterized by values of G' , G'' (Pa) as a function of frequency in the range of 0.01–10 Hz and temperature of 25 °C. The storage modulus can be used as a measure of the elastic component of the sample and similarly, the loss modulus – the viscous component of the sample.

2.11. Statistical analysis

The experimental results were analyzed by one-way analysis of variance (ANOVA) using SPSS 16 (SPSS Inc., Chicago, IL). The comparison of treatment means was carried out using Duncan's multiple range test.

3. Results and discussions

3.1. Chemical composition

Chemical analysis and composition of QSG were presented in Table 1. On the average, the gum contains $85.04 \pm 2.87\%$ carbohydrate, $13.16 \pm 1.73\%$ uronic acid, $5.77 \pm 0.83\%$ moisture, $2.78 \pm 0.21\%$ protein, $5.64 \pm 0.21\%$ ash, and $0.75 \pm 0.09\%$ fat. Carbohydrates constitute the major component of the extracted QSG. Comparatively, the carbohydrate content of QSG was close to that reported for locust bean gum (85.1-88.7%) and was more when compared to those reported for some commercial gums like guar gum (71.1%), and gum ghatti (78.36%)⁶.

The monosaccharide composition in Table 1 showed that xylose (40.43 % of total carbohydrates) was the primary saccharide component followed by mannose (31.11% of total carbohydrates) with minor amounts of other monosaccharides (Table 1). The high amount of xylose and mannose in QSG composition may indicate that the structural make-up of this gum can consist of xylan and/or mannan backbone with the branching of arabinose, galactose, and glucose.

The yield of the extracted gum from quince seed was found to be 9.63%, which was approximately close to that reported in the previous study (10.9%)²⁰. However, they also reported that QSG had 20.9% protein which indicates that the purified QSG in their research has a low degree of purity. In another research, the gum was extracted from quince seed

without purification yielding the yield value of 11.58%⁴. In general, due to high carbohydrate content (85.04%) and high level of extraction yield (9.63%), it can be concluded that this gum is an appropriate alternative for commercial gums.

The amount of protein in the polysaccharide composition is a determinant factor in food and pharmaceutical systems. For example, several properties of gum such as a film forming capacity, emulsifying and stabilizing features as well as foaming properties are dependent on protein fraction⁶. Comparatively, the protein content of QSG in this study was 2.78 % which is comparable to that cited for xanthan gum (2.125%), and less than those reported for locust bean gum (5.2-7.4%), and guar gum (8.19%)^{21, 22}. The presence of proteins in the QSG composition reflects the ability of this biopolymer to reduce surface tension²³.

Uronic acid content is an indicator of the relative degree of polyelectrolyte nature and amount of acidic polysaccharides²⁴. The uronic acid content of QSG (13.16 %) was less than those of flaxseed gum (21.0-25.1%) and xanthan gum (21.5%) and was more than those reported for gum ghatti (12.83%), and gum Arabic (15.0%)⁶. Therefore, QSG has more negative charge than ghatti and Arabic gums. Based on the previous finding, the gums with a high amount of acidic polysaccharide indicate typical Newtonian-like behavior²¹.

Ash content of QSG samples was found to be 5.64 % which is in the range of other natural polymers. Comparatively, the ash content of QSG was more than those of locust bean gum (0.7-1.5%), Arabic gum (1.2 %) and xanthan gum (1.5%), and was less than that of guar gum (11.9%)^{21, 22}.

The results of chemical analysis observed here were different from those reported in previous research^{4, 20}. Different factors can influence on chemical and functional properties of hydrocolloids like source, extraction and purification processes and growing conditions¹. It can

be suggested that the purification technique applied in the current research could be the main reason for this discrepancy in chemical composition.

Mineral profile of gums can influence on some their physicochemical characteristics such as emulsifying properties (iron) ²³, viscosity-enhancing feature and gel-forming ability of polyelectrolyte gums (Ca⁺²) ²⁵, and enzyme activity (Ca²⁺ and Mg²⁺). Additionally, some minerals are essential for human health, whereas some of them like copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd), and arsenic (As) may also have adverse effects at high concentration. Therefore, mineral composition of QSG was determined. The mineral composition of QSG is summarized in Table 1. QSG is gum with a considerable nutrient value due to the presence of high amount of calcium (7331.1 ppm), and magnesium (2632.8 ppm). Based on this result, this gum can be introduced as a value-added by-product in the food and pharmaceutical industries.

(Table 1)

3.2. FTIR analysis

FT-IR analysis of QSG was carried out to identify the organic functional groups in gum structure. The FT-IR analysis of QSG is consistent with the compositional analysis described above. FTIR spectrum of QSG is presented in Fig. 1, which illustrates all typical bonds and peaks characteristic of the polysaccharide. The region between below 700 cm⁻¹ is related to the skeleton vibration of the gum with two modes consisting of angle deformation formed by heavy atoms (CCO, COC) and internal rotations around CO bonds ²⁶. The peaks diagnosed between 800 to 1200 cm⁻¹ is called fingerprint or anomeric area for carbohydrates. This region is sensitive to conformational and configurational transformations of polysaccharides ²⁷ and can be used as a measure of structural differences in the various polymers. For example, Guiliano, Asia, Chatel

and Artaud²⁸ differentiated *Acacia Senegal* gum and *Acacia seyal* using the relative intensities of this region. The signal at 897.70 cm^{-1} is evidence for the presence of β -D-glycosidic linkages types of the aldopyranose in biopolymer structure²⁹. It also suggested that the peak at 1025 cm^{-1} is due to the presence of uronic acid in the polysaccharide structure³⁰. Accordingly, it can be found that QSG is a polysaccharide comprising uronic acid which was supported by the result observed for proximate analysis observed above.

The observed signals between 950 and 1200 cm^{-1} refer to stretching vibration of alcoholic C-O in COH bands in carbohydrates³¹. The range between 1200 - 1500 cm^{-1} results from coupling of the deformation vibrations of groups containing hydrogen atoms, namely CCH, HCH, COH and HCO²⁷. As it can be seen in Fig. 1, these bands in this range were 1249.61 , 1375.02 , and 1424.40 , cm^{-1} . The peak observed at 2920 cm^{-1} is related to C-H stretching, symmetric and asymmetric of the free sugar and also the vibration of symmetric stretching of CH_2 , indicating the existence of grafting³². This peak also could be assigned to the doubles overlapping with O-H³³.

The wave numbers between 3000 to 3500 cm^{-1} are referred to free, inter, and intramolecular bond O-H groups³⁴, and also reveals several properties such as free O-H groups stretching bonds that occur in the vapor phase and bonded with hydroxyl bands in the structure of carboxylic acid³⁵. Therefore, it can be realized that this gum contained carboxyl groups, which can act as a binding site for ions and as a result leads to improve viscous-enhancing properties and gel-forming capacity. Additionally, Sharma and Mazumdar³⁶ stated that this broad absorbance peak is related to hydrogen bonding involving the O-H groups of glucopyranose ring in the structure of polysaccharide chain.

3.3. NMR analysis

In order to investigate the structure of QSG, NMR analysis was conducted. The ^{13}C NMR spectrum of this gum indicated it has a highly branched structure. The spectrum of QSG contained the signals corresponded to $(1\rightarrow4)\rightarrow\beta\text{-D-Xylp}$, $(1\rightarrow4)\text{-}\beta\text{-D-Xylp-2-o-GlcpA}$, and $4\text{-o-Me-}\alpha\text{-D-Glcp}$ (Table 2). The resonance for carbonyl groups of the terminal non-reducing D-glucuronic acid was observed at 176.17 ppm³⁷. The anomeric region includes various glycoside linkages in polysaccharide structure. The peak observed at 97.52 ppm is related to $4\text{-o-Me-}\alpha\text{-D-Glcp}$ ($1\leftarrow$ residues and the main one at 101.64 ppm is due to $(1\rightarrow4)\text{-}\beta\text{-D-Xylp-2-o-GlcpA}$. The peak at 104.19 is related to $(1\rightarrow4)\rightarrow\beta\text{-D-Xylp}$. Therefore, it can be suggested that QSG has a highly substituted xylan structure. Additionally, the signal at 109.53 ppm and 62.21 ppm arise from C-1 and C-2 of residual $\alpha\text{-L-Araf}$ units.

(Fig. 2)

(Table 2)

3.4. Analysis of macromolecular weight and its characteristics

The molecular weight of a biopolymer is considered as an important parameter due to its effect on functional characteristics of polysaccharides³⁸. Therefore, molecular properties of QSG were analyzed. GPC elution profile of QSG is depicted in Fig. 3. Weight average molecular weight of QSG (9.61×10^6 g/mol) was more when compared to commercial gums such as xanthan gum (4.05×10^6 Da)³⁹, guar gum (1.45×10^6 Da)⁴⁰, gellan gum (1.64×10^6 Da)⁴¹ and locust bean gum (1.6×10^6 Da)⁴². The molecular weight is a determinant factor for thickening and gelling properties of hydrocolloids. For example, xanthan has a molecular weight (4200 kDa) and as a result impart a high level of viscosity-enhancing property^{43, 44}. Therefore, QSG can be

introduced as a good thickening agent because it has high molecular weight. Rheological properties of QSG will be discussed in the continuation of this research. Moreover, high molecular weight polysaccharide does not have much tendency to be adsorbed at the air-water interface, but they can profoundly reinforce the protein foams stability due to thickening or gelling characteristics⁴⁵. Thus, QSG can be introduced as a good stabilizing agent in protein foams. Number average molecular weight (M_n) of QSG (4.153×10^6 g/mol) was more when compared to most hydrocolloids that have been studied so far.

(Fig. 3)

3.5. Rheological properties

3.5.1. Dilute solution properties

Before intrinsic viscosity measurements, master curve ($\log \eta_{sp}$ against $\log C \times$ intrinsic viscosity) was plotted to confirm that tested solutions are in a dilute domain. When the slope of the master curve is less than 1.4, it can be found that there are no coil overlaps. In a concentrated regime, the slope increase to 3.3⁴⁶. The results indicated that slopes of master curves for QSG at salt concentration were in the range of 0.98 to 1.99, exhibiting that QSG solutions were in the dilute domain without molecular entanglements.

Intrinsic viscosity is a property related to the hydrodynamic volume occupied by the polymer²¹. The high determination coefficient and low mean square error revealed that Tanglertpaibul-Rao's equation might be adequate to estimate intrinsic viscosity of QSG (Fig. 4). This result is in line with those reported in previous studies^{23, 46-49}. The intrinsic viscosity of QSG was 16.96 dl/g (in deionized water at 25 °C) which was more than the data obtained for gum arabic (0.6 dl/g at

20 °C) and guar gum (9.25 dl/g), and smaller than that estimated for Tragacanth gum (19.2 dl/g),
6.

Following an increase in the ion concentration, the intrinsic viscosity of QSG decreased which is due to the shielding influence of charges on macromolecular chains⁵⁰. With increasing the ion concentration, the impact of shielding of charges reinforced and consequently the molecular repulsions, followed by intrinsic viscosity diminished⁵¹. The similar results have been reported for sodium alginate⁵², Balangu seed gum⁵¹, xanthan¹⁴, *Prunus armeniaca*⁴⁹ and hsiantSao leaf gum¹⁶.

Salt tolerance parameter is related to polymer chain stiffness. The less ionic strength dependency of intrinsic viscosity demonstrates a high level of chain flexibility¹⁹. The value of relative chain stiffness (B) of QSG in the presence of CaCl₂ (0.24) was less than those of sage seed gum (0.381)⁵³ and Balangu seed gum (0.346)⁵¹. Accordingly, QSG has a stiffer conformation in comparison to these biopolymers. On the other hand, the value obtained for B number was more than that of reported for rigid helices gum like xanthan (0.00525)⁵⁴.

(Table 3)

(Fig. 4)

3.5.2. Steady shear behavior

Hydrocolloids foods are occasionally processed under different environmental conditions such as mineralized solutions. Hence, the evaluation of salt addition influence on rheological properties of QSG is important to appointment mucilage function as a polyelectrolyte. The viscosity of

charged macromolecules is considerably affected by ionic strength. In the absence of counter ions, a negative charge reinforces the electrostatic repulsion and as a result, produces a more expanded molecule. The results of the compositional analysis indicated that QSG had negatively charged polyelectrolyte molecules. Therefore, it is expectable that this gum can produce a high viscous solution in deionized water, because of high repulsive forces. The effect of CaCl_2 addition on consistency coefficient of QSG solution is summarized in Table 3. The steady shear flow behavior of QSG solutions at all tested ion concentrations can be described by non-Newtonian profile characterized by a flow behavior index less than one. Following an increase in ionic strength from 0 to 50 mM, the consistency coefficient (as a measure of apparent viscosity) decreased. The reason for that may be related to decreasing in the electrostatic repulsion and molecules expansion which leads to a reduction inconsistency coefficient⁵⁵. Furthermore, inorganic cations have a high degree of hydration and as a result of compactness of the hydrated layer around polyelectrolyte molecules. Therefore, an increase in ionic strength led to a decrease in consistency coefficient of solutions⁵⁶. These observations are consistent with those of hsian-tsau leaf gum⁵⁷ and *Opuntia ficus-indica* gum⁵⁵. Surprisingly, when the CaCl_2 concentration increased from 100 to 500 mM, the magnitude of consistency coefficient increased from 12.21 to 14.88 $\text{Pa}\cdot\text{s}^n$, indicating CaCl_2 can reinforce the structural network of QSG. The experimental data of log apparent viscosity vs log shear rate is also presented as Fig. 5 which confirmed the effect of CaCl_2 on steady shear behavior of QSG.

With increasing salt concentration from 0 to 50 mM, flow behavior index increased from 0.28 to 0.30, indicating that hydrocolloid solutions tend to be lower shear thinning in the presence of salt. On the other hand, with further increasing of salt concentration, flow behavior index values

decreased. This trend may be due to the reinforcement of electrostatic interaction between calcium ions and polymer macromolecules and as a result increasing molecular entanglements.

(Table 3)

(Fig. 5)

3.5.3. Dynamic rheological behavior

3.5.3.1. Strain sweep measurements

With increasing strain, two distinct domains namely linear viscoelastic region where G' and G'' were almost constant, and nonlinear region in which G' and G'' started to decrease were distinguished. In the strain sweep tests, G' remained constant until the strain reached a critical point at which G' started to decrease sharply, as demonstrated in Fig. 6-a. The strain at which G' decreased sharply is defined as the critical strain. Therefore, critical strain reflects the deformability of the gum⁵⁸. Strong gum solutions maintain longer at linear state compared to weak gum solutions⁵⁹; in other words, viscoelastic moduli can be linear in a wide strain range.

The linear region for dilute solutions is less than concentrate solutions and this is less than gels.

While the strain value at the limit of LVE rarely exceeds 0.1 for colloidal gels, a larger LVE region with a strain equal to or exceeds 1 is for natural biopolymer gels⁶⁰.

(Fig. 6)

At low salt concentrations (10–50 mM), the elastic modulus remained constant at strain up to about 20%. With increasing in salt concentration, the strain at which the elastic modulus decreases, increased to more than 100%. While in the absence of salt, elastic modulus remained stable at strain up to about 3% (Table 3), which indicates that increasing salt concentration

increased the strength of the system and got more rigid. The values of G' and G'' at LVE region also increased with increase in salt concentrations (Table 3). Increasing salt concentration from 10 to 500 mM increased the structural strength (G' at LVE) of gum solution at constant concentration. It should be noted that, at low salt concentrations (10 – 50 mM), compared to the absence of salt, the values of G' and G'' were decreased. This shows that at low concentrations of salt, structural strength (G' at LVE) of gum solution was weakened. This kind of experiment also reveals the upmost deformation that a system can tolerate without structural failure; in another word, determination of the critical strain as corresponding of the linear viscoelastic range is a yardstick of structural strength and shape maintenance factor versus the mechanical stresses⁵⁸. The critical strain values for QSG were 3.7, 74.5, 75.1, 110.6 and 131.1% for 0, 10, 50, 100 and 500 mM salt concentrations, respectively. At this point, G' diminished aggressively, which exhibits the strain that led to the first non-linear changes in the structure of aqueous gum dispersion.

3.5.3.2. Frequency sweep measurements

Frequency sweep data can be applied to determine or categorize dispersions. The four most ordinary and common classifications are that of a dilute solution, an entanglement network systems (or a concentrated solution), a weak gel and a strong gel⁶⁰. Fig 6-b shows the frequency dependence of G' for QSG in the presence of Ca^{2+} ions at ambient temperature. At any concentration of CaCl_2 from 0 mM to 500 mM, G' was larger than G'' (Data not shown), and both moduli were only slightly frequency dependent throughout the accessible frequency range. This behavior is classified rheologically as that of a weak gel^{61, 62}. Therefore, the gel-like

behavior was observed at all various concentrations of CaCl_2 . According to Martínez-Ruvalcaba, Chornet and Rodrigue⁶³, in weak gels, both moduli display light frequency dependency, with G' exceeding G'' at all frequencies. However, G' and G'' values for 10 and 50 mM CaCl_2 concentrations were lower than that of the blank sample without salt (Fig. 6), showing that the elastic properties can be decreased at the special concentration. This result is consistent with that observed in steady shear behavior.

4. Conclusion

QSG is a high molecular weight polysaccharide contains 6.39% arabinose, 40.43% xylose, 5.60% galactose, 5.75% glucose and 31.11% mannose. By using FT-IR and ^{13}C NMR analysis, the characteristics of functional groups and structural properties of QSG were identified. The results of monosaccharide composition and ^{13}C NMR analysis showed that the structural make-up of this gum could consist of xylan and/or mannan backbone with the branching of arabinose, galactose, and glucose. FT-IR analysis indicated the presence of carboxyl group, which can

improve rheological properties of QSG. The dilute solution behavior of QSG in the presence of CaCl_2 was evaluated. The value of salt tolerance parameter of QSG exhibited that this gum has a stiff conformation. Based on the steady and dynamic behavior of QSG, it was found that rheological properties of this gum improved in the special concentration of CaCl_2 .

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Table 1. Chemical composition of QSG*

Composition (%)	QSG*
Carbohydrate	85.04±2.87
Monosaccharide	
L-Arabinose	6.39±0.18
Rhamnose	<MDL **
D-Xylose	40.43±0.47
D-Galactose	5.60±0.08
D-Glucose	5.75±0.08
D-Mannose	31.11±0.48
Galacturonic acid	0.81±0.08
Glucuronic acid	8.54±0.20
Uronic acid	13.16±1.73
Protein	2.78±0.21
Moisture	5.77±0.83
Fat	0.75±0.11
Ash	5.64±0.48
Elements (ppm)	
Calcium (Ca)	7333.1 ± 1.32
Magnesium (Mg)	2632.8 ± 2.21
Manganese (Mn)	11.2 ± 0.21
Potassium (K)	74.12 ± 1.40
Zinc (Zn)	96.03 ± 2.00
Cobalt (Co)	<MDL
Copper (Cu)	2.01 ± 0.08
Aluminum (Al)	2.28 ± 0.44
Nickel (Ni)	0.06 ± 0.01
Lead (Pb)	<MDL
Cadmium (Cd)	<MDL
Arsenic (As)	<MDL

*Values are means ± SD of triplicate determination

**MDL: Method detection limits

Table 2. ^{13}C signals of QSG. Chemical shift is expressed in ppm.

Residue	C-1	C-2	C-3	C-4	C-5	C-6	O-CH ₃
(1→4)→β-D-Xylp	104.79	72.27	71.94	73.47	6.21	-	-
(1→4)-β-D-Xylp-2-o-GlcpA	101.64	75.11	71.02	73.17	62.16	-	-
4-o-Me-α-D-Glcp	97.52	70.67	75.26	81.49	71.26	176.17	59.29

Table 3. The effect of ionic strength on steady shear parameters of QSG solutions (0.5%, at 25 °C) and viscoelastic properties (quantified by strain sweep experiments at frequency of 1 Hz).

Steady shear test	k (Pa s ⁿ)	n (-)	R^2	RMSE
CaCl ₂ (mM)				
0	7.62 ± 0.44 ^c	0.28 ± 0.00 ^{ab}	0.999	0.000
10	6.11 ± 0.33 ^d	0.29 ± 0.01 ^a	0.999	0.003
50	6.01 ± 0.23 ^{cd}	0.30 ± 0.01 ^a	0.998	0.009
100	12.21 ± 0.18 ^b	0.21 ± 0.01 ^c	0.998	0.011
500	14.88 ± 0.96 ^a	0.20 ± 0.02 ^c	0.994	0.012
Strain sweep test	G'_{LVR} (Pa)	G''_{LVR} (Pa)	γ_{LVR} (%)	Tan δ_{LVR}
0	15.01±0.26 ^a	6.94±0.19 ^a	3.7±0.35 ^d	0.46±0.01 ^b
10	4.97±0.67 ^d	2.62±0.14 ^b	74.5±1.18 ^c	0.53±0.01 ^a
50	5.10±0.56 ^d	2.16±0.23 ^e	75.1±4.17 ^c	0.42±0.02 ^c
100	12.69±0.23 ^c	3.77±0.11 ^d	110.6±3.12 ^b	0.29±0.01 ^d
500	14.32±0.22 ^b	4.36±0.22 ^c	131.1±6.14 ^a	0.30±0.02 ^d

a, b, c, d, and e Different letters in the same column indicate significant differences at 5%.

Figures caption

Fig. 1 FT-IR spectrum of QSG.

Fig.2. ^{13}C NMR spectrum of QSG.

Fig. 3 Molecular properties of QSG.

Fig. 4 The plot of relative viscosity against solution concentration

Fig.5 The experimental data of log apparent viscosity vs log shear rate

Fig. 6 Changes of storage modulus in strain sweep (a) and frequency sweep (b) tests at various ion concentrations.

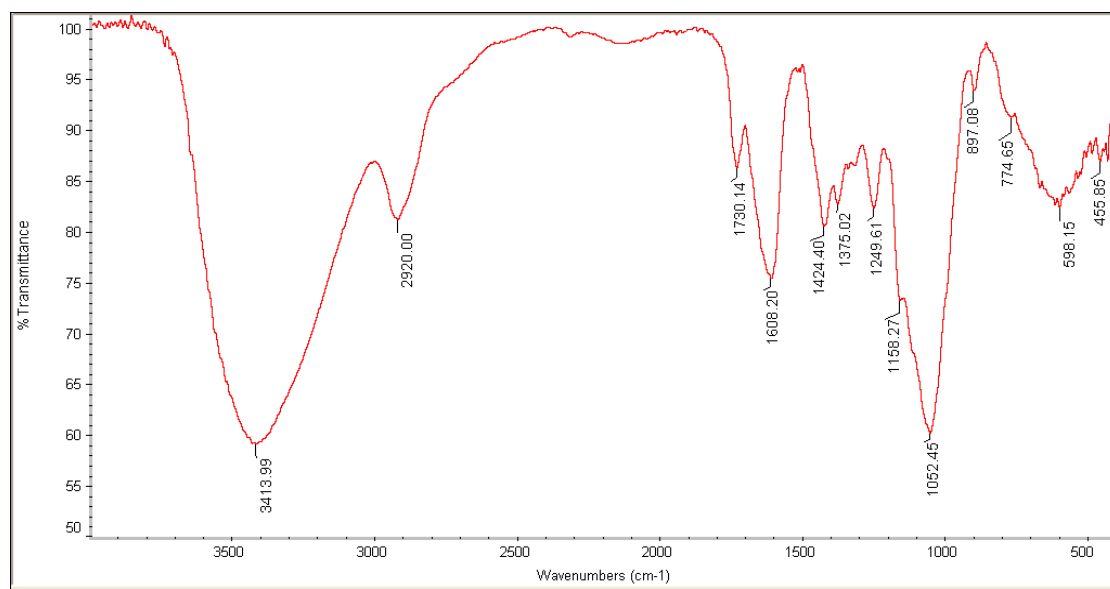


Fig. 1

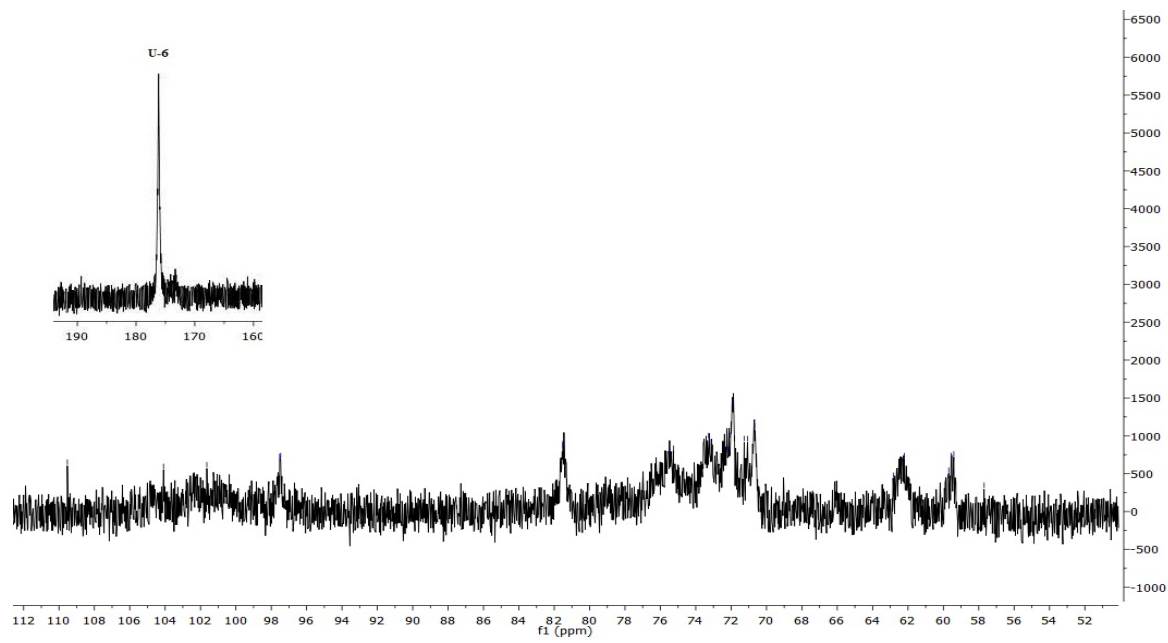


Fig. 2

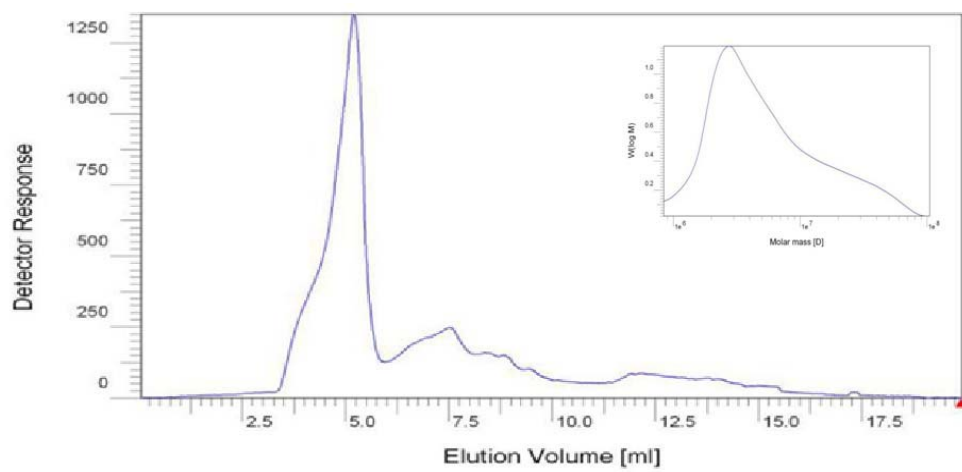


Fig. 3

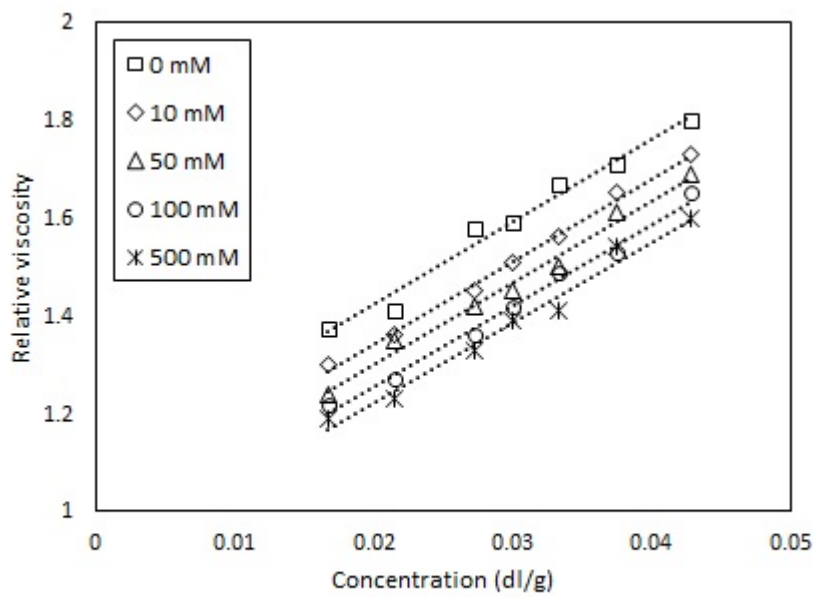


Fig. 4

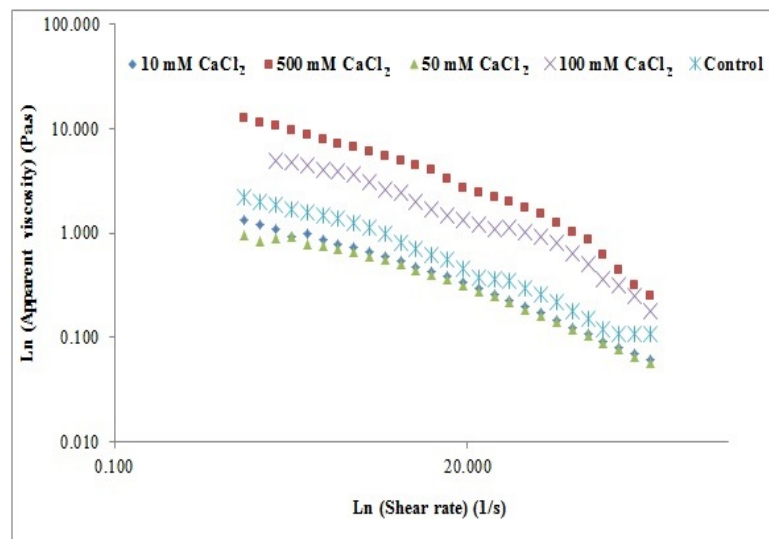


Fig. 5

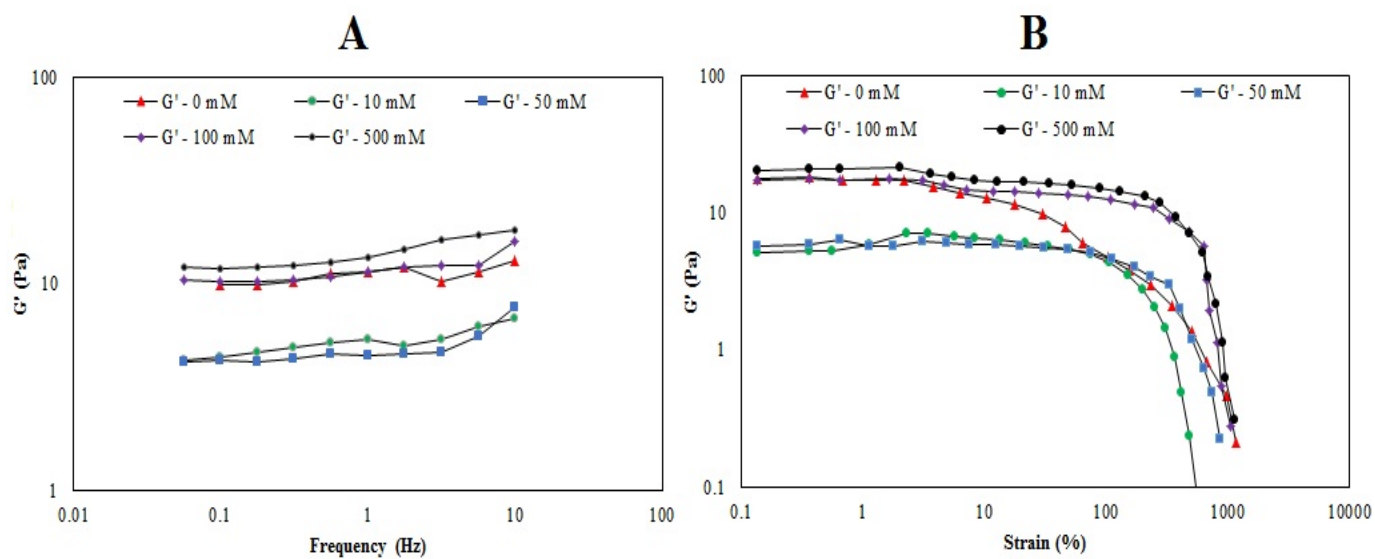


Fig. 6