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Introducing *Prunus cerasus* gum exudates: Chemical structure, molecular weight, and rheological properties

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ABSTRACT

In this work, some physicochemical characteristics of *Prunus cerasus* gum exudates (PCGE) were evaluated. Ash, moisture, protein, fat and carbohydrate contents were found to be 2.8, 6.2, 2.4, 1.48 and 71.51%, respectively. PCGE consisted of L-arabinose, D-galactose, xylose, mannose and rhamnose in a 31.31:24.04:21.31:17.63:5.69 molar percentages. FTIR spectrum showed the presence of carboxyl, hydroxyl and methyl groups and glycoside bonds. ^{13}C NMR and ^{1}H NMR analysis suggested an arabinogalactan structure for this gum. The weight average molecular weight (1.119×10^7 g/mol), the number average molecular weight (4.153×10^6 g/mol) and polydispersity index (2.69) were determined. Intrinsic viscosity for PCGE in deionized water was 3.7 dl/g according to Kraemer equation. Steady shear measurements as a function of concentration (4, 6 and 8%) showed that PCGE solutions had shear thinning behaviour. Oscillatory shear experiments demonstrated that in all tested concentrations (4, 6 and 8%), loss modulus (G″) is superior to storage modulus (G′), indicating that the solutions demonstrate higher viscous behaviour than a clear tendency to form a gel. Colour parameters including lightness (84 ± 0.219), redness (2.048 ± 0.043), yellowness (21.962 ± 0.088) and chroma (22.057 ± 0.12) were determined.

1. Introduction

Natural gums as biological macromolecules are extensively used in food industry as emulsifier, stabilizer, flavour encapsulator, edible coating, and thickener. In comparison to synthetic and semi-synthetic polymers, natural polymers have many advantages such as being non-toxic, sustainable, biodegradable, biocompatible, eco-friendly and easy access (Singh, Kumar, Langyan, & Ahuja, 2009).

Plant gum exudates are produced by a phenomenon called gummosis. These exudates obtain from branches of trees in place of mechanical and microbial injury. The ever increasing interest in gum exudates is because of their structural diversity and metabolic functions in food and pharmaceutical systems (Nishinari, Zhang, & Ikeda, 2000). *Prunus cerasus* gum is a tree exudates gum that belongs to the *Rosaceae* family. Some physicochemical characteristics of *Prunus* gums from *Rosaceae* family such as *Prunus cerasoides D. Don.*, *Prunus amygdale*, *Prunus domestica* L., *Prunus persica*, *Prunus armeniaca* and *Prunus dulcis* have been evaluated (Bouaziz et al., 2015; Fathi, Mohebbi & Koocheki, 2016; Mahfoudhi, Chouaibi, Donsì, Ferrari, & Hamdi, 2012; Malsawmtluangi et al., 2014; Petrea, Amarioarei, Apostolescu, Puiel & Ciovica, 2013; Simas et al., 2008).

Gum functional characteristics depend on physicochemical properties. The objectives of this paper were, therefore, to investigate the chemical composition, molecular structure, molecular weight, steady shear and viscoelastic behaviour, intrinsic viscosity and colour properties of PCGE as a starting point for evaluating the potential use of this gum in food and pharmaceutical industries.

2. Material and method

2.1. Materials

PCGE samples were obtained from *Prunus cerasus* trees in Bojnord, Rooin during the month may (spring). Geographically, Rooin is in 37° 12 min 13.9 s North and 57° 29 min 13.5 s East. The gum samples were cleaned to remove all impure matters. The samples were stored in airtight polypropylene jars. The entire chemicals were purchased from Merck (Darmstadt, Germany).

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2.2. Purification of PCGE

Purification of the PCGE was performed based on Fathi et al. method which was earlier described (Fathi et al., 2016). Briefly, the native exudates were dissolved with deionized water and centrifuged (3000*g*, 20 min, 20 °C) to separate any bubbles and insoluble materials. The soluble materials were precipitated using acetonic precipitation. The resulting precipitate was freeze dried and used for the experiments.

2.3. Analytical methods

2.3.1. Moisture, ash, nitrogen, and fat contents

Moisture content of PCGE samples was measured by AOAC method at 105 °C until constant weight was obtained, and then, moisture content was calculated as follows:

$$\%\,\text{Moisture content} = \frac{\text{Weight of water in sample}}{\text{Total weight of wet sample}} \times 100 \qquad (1)$$

Nitrogen, ash, and fat contents were quantified according to the AOAC method in which nitrogen to protein conversion factor of 6.25 was applied to calculate crude protein content (AOAC, 1990).

Carbohydrate content was determined by phenol-sulphuric acid method. Uronic acid content was analyzed by carbazole method using D-galactose as standard (Brummer & Cui, 2005). All the experiments were done in triplicate and the average values were reported.

2.3.2. Elemental analysis

The content of some minerals was measured using the earlier reported method (Lima, Barbosa, Krug & Tavares, 2002). First, an amount of 0.5 g PCGE samples was weighted and then nitric acid and hydrogen peroxide (2:1) were added to PFA digestion vessel. Decomposition was performed using a microwave oven at 400 W for 15 min. Finally, samples were cooled to 25 °C and the volume made up with deionized water.

Elemental analysis was performed using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (SPECTRO ARCOS, Ametek. Germany). ICP-OES operating parameters for the analysis were as follow: 1400 W; plasma Ar gas flow: 12 l/min; auxiliary: 0.5 l/min; nebulizer flow: 0.1 l/min; sample flow rate: 1.4 ml/min; signal processing: peak area-3 pixel; pump speed: 300 rpm; replicate measurements: 3.

2.3.3. GC-MS analysis

GC-MS analysis was done according to the method given by Fathi et al. (2016). Briefly, PCGE powder (1 g) was hydrolyzed with 2 M TFA for 8 h at 100 °C. The first step of the alditol acetate derivatization procedure was performed for reduction of monosaccharides to their corresponding alditols. For this purpose, the reduction of the acid hydrolyzed PCGE was carried out with $NaBH_4$ (Wolfrom & Thompson, 1963). For acetylation of alditols, acetic anhydride (500 μL) and pyridine (500 μL) were added and incubated for 20 min at 100 °C. In the final step, in order to extract alditol acetate, 500 μL of ethyl acetate and 2 ml of water were added. To obtain clear separate layers (ethyl acetate on top, water on bottom), centrifugation (2000 RPM for 5 min) was carried out. Ethyl acetate layer was evaluated using Agilent gas chromatograph fitted with HP-5MS capillary column (30 m length, 0.25 mm diameter, and 0.25 μm film thickness). Helium was utilized as the carrier gas at a flow rate of 1.3 ml min^{-1}. The injector and MS source temperatures were 280 and 230 °C, respectively. The oven temperature was programmed from temperature 120 °C (hold time = 2 min), increased to 300 °C at the rate of 6 °C min^{-1}, followed hold at 300 °C for 15 min.

2.3.4. ^{13}C and ^{1}H nuclear magnetic spectroscopy (NMR)

For NMR spectroscopy, an amount of 5–10 mg PCGE sample was dissolved in 0.5 ml D_2O in 5 mm tube. ^{13}C and ^{1}H NMR spectra of PCGE sample were recorded at 25 °C using a Bruker DRX Avance spectrometer.

2.3.5. FTIR analysis

The major functional groups of the sample were analyzed using a FT-IR spectroscopy (AVATAR 370 FT-IR, Thermonicolet). Until the time of analysis, PCGE powder was stored in desiccators with silica gel. The percent transmittance was recorded in the absorbance mode from 400 to 4000 cm^2 at a resolution of 4 cm^2.

2.3.6. Molecular weight determination

Molecular weight averages (Mn and Mw) of PCGE were determined by gel permeation chromatography (GPC) using a PL Aquagel-OH Mixed-H column. The sample was dissolved in deionized water, de-dusted by passing through a 0.2 μm filter and then injected at a constant flow rate of 1 ml/min. The eluent water was used and monitored with refractive index detector. For calibration, a standard curve was constructed using dextran molecular weight standards with Mw between 5200 and 988,000 g/mol.

2.4. Rheological behaviour

2.4.1. Intrinsic viscosity determination

For measuring the intrinsic viscosity of PCGE solutions, a stock solution (0.3 g dL^{-1}) was prepared using deionized water. Intrinsic viscosity measurement was done at 25 °C ± 0.1 °C, using an Ubbelohde capillary viscometer (Cannon instrument Co., USA), which is suspended in a precision water bath to control the desired temperature. At least three efflux times at each concentration were observed. In order to convert the sample viscosity (η) to relative viscosity (η_{rel}) and specific viscosity (η_{sp}) following equations were utilized:

$$\eta_{rel} = \frac{\eta}{\eta_{sp}} \qquad (2)$$

$$\eta_{sp} = \frac{\eta - \eta_{sp}}{\eta_{sp}} = \eta_{rel-1} \qquad (3)$$

where, η_{sp} is the viscosity of the solvent (deionized water).

Measurements were carried out at different concentrations and the value of intrinsic viscosity was calculated from the extrapolation of ln η_{rel}/c or η_{sp}/c to infinite dilution based on Huggins and Kraemer equations (Eqs. (4) and (5)).

Huggins equation (Huggins, 1942):

$$\frac{\eta_{sp}}{C} = [\eta] + k_1[\eta]^2C \qquad (4)$$

Kraemer equation (Kraemer, 1938):

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k_2[\eta]^2C \qquad (5)$$

here, k_1,k_2 and C are the Huggins constant, Kraemer constant and the polymer concentration, respectively.

Tanglertpaibul & Rao (1987) measured the intrinsic viscosity by calculating the slope of relative viscosity or specific viscosity vs. concentration based on the following formula:

$$\eta_{rel} = 1 + [\eta]\,C \qquad (6)$$

In addition, Higiro's equations were used for estimating intrinsic viscosity of PCGE as follow (Higiro, Herald, & Alavi, 2006):

$$\eta_{rel} = e^{[\eta]C} \tag{7}$$

$$\eta_{rel} = \frac{1}{1-[\eta]C} \tag{8}$$

Berry number was quantified from the slope of a double logarithmic plot of η_{sp} vs. concentration as follow:

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

Parameter b can be utilized as a useful parameter for evaluating the conformation of a polysaccharide (Lai, Tung, & Lin, 2000).

The relation between intrinsic viscosity and two molecular parameters, known as voluminosity and shape factor of the polymer coil can be evaluated as the following relation:

$$[\eta] = \nu V_E \tag{10}$$

here, ν and V_E are known as shape factor and swollen specific volume or voluminosity, respectively. Shape factor is expressed as an anhydrous macromolecule will essentially expand when dissolved in solution because of solvent association, and voluminosity (which indicate the volume of the macromolecule in solution per unit anhydrous mass of macromolecules) is a useful parameter for estimating the conformation of a polymer in various solvent conditions which could be quantified through intercept of plotting Y value vs. C as follow (Antoniou, Themistou, Sarkar, Tsianou, & Alexandridis, 2010; Lai et al., 2000).

$$Y = \frac{\eta_{rel}^{0.5} - 1}{\left[C\left(1.35\,\eta_{rel}^{0.5} - 0.1\right)\right]} \tag{11}$$

Using Eq (10), we can quantify shape factor value for PCGE molecules.

Based on the Einstein viscosity relation, Antoniou et al. (2010) indicated that hydrodynamic coil radius, R_{coil}, can be estimated using the following equation:

$$R_{coil} = \left[\frac{[3[\eta] \times M_W]}{10\pi \times N_{AV}}\right]^{1/3} \tag{12}$$

here, M_W and N_{AV} are the polymer weight average molecular weight and Avogadro's number ($6.022 \times 10^{23}\,\mathrm{mol}^{-1}$), respectively.

Coil volume (V_{coil}) can be determined by the following equation if shape of the coil be assumed to be sphere like:

$$V_{coil} = \frac{4}{3}\pi R_{Coil}^{3} \tag{13}$$

2.4.2. *Steady shear measurements*

Examination of the rheological properties of the PCGE solutions was performed by a rotational viscometer (Model RVDV-II, Brookfield Engineering, Inc., USA) equipped with a heating circulator (Julabo, Model F12-MC, Julabo Labortechnik, Germany) at 25 °C using an SC4-31 spindle. Three concentration levels of the PCGE solution (4, 6 and 8%) were prepared at room temperature. The samples were dissolved in deionized water at 300 rpm and kept at 4 °C for over night to hydrate completely. The steady shear flow measurement was done over a wide range of shear rate from 10 to 300 s^{-1}.

Consistency coefficient and flow behaviour index values were determined using the power law model:

$$\tau = k\dot{y}^{n} \tag{14}$$

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 behaviour index (dimensionless). All the measurements were carried out at 25 °C.

2.4.3. *Oscillatory measurements*

Dynamic oscillatory shear measurements were done as a function of PCGE concentration (4, 6 and 8% (w/w)) using a controlled rheometer (Malvern Bohlin Gemini) fitted with a cone-plate device (cone angle: 4°: diameter: 40 mm: gap: 150 μm). Strain sweep experiments were first done on the PCGE solutions at a constant frequency to distinguish the linear viscoelastic region. Frequency sweep measurements at a constant strain in the linear viscoelastic region (LVR) were carried out to determine the viscoelastic nature of PCGE. The mechanical spectra recorded were evaluated by values of G′ and G″ (Pa) as a function of frequency (f). All the measurements were performed at 25 °C.

2.5. *Colour measurement*

In order to determine colour parameters of PCGE samples such as L* (lightness), a* (redness) and b* (yellowness) a Chroma-meter (Chroma Meter model CR-410, Minolta Co. Ltd., Osaka, Japan) was used. It was standardized using black and white ceramic plate. CIE (Commission International de l'Eclairage) L*, a* and b* were measured from five surfaces of the ground PCGE powder samples. The instrument was set at illuminant D-65 and a 2° observer angle.

Table 1
Chemical composition of *Prunus cerasus* gum exudates[a].

Composition (%)		PCGE
Protein		2.40 ± 0.10
Carbohydrate		71.51 ± 0.02
Uronic acid		7.31 ± 0.54
Ash		2.80 ± 0.01
Moisture		6.20 ± 0.15
Fat		1.48 ± 0.13
Monosaccharides		–
	Arabinose	31.31 ± 0.39
	Galactose	24.04 ± 0.89
	Xylose	21.31 ± 0.23
	Mannose	17.63 ± 0.96
	Rhamnose	5.69 ± 0.69
Elements (ppm)		
	Calcium (Ca)	5200.2 ± 0.97
	Iron (Fe)	552.3 ± 1.70
	Sodium (Na)	264.3 ± 1.40
	Phosphorus (P)	1676.5 ± 1.32
	Selenium (Se)	<MDL
	Magnesium (Mg)	2840 ± 4.00
	Manganese (Mn)	19.2 ± 0.00
	Potassium (K)	204.5 ± 1.40
	Zinc (Zn)	106.8 ± 0.10
	Cobalt (Co)	<MDL[b]
	Chromium (Cr)	3.7 ± 0.02
	Copper (Cu)	0.5 ± 0.000
	Tin (Sn)	<MDL
	Silver (Ag)	<MDL
	Aluminum (Al)	8.38 ± 0.50
	Nickel (Ni)	0.03 ± 0.00
	Lead (Pb)	<MDL
	Mercury (Hg)	<MDL
	Cadmium (Cd)	<MDL
	Arsenic (As)	<MDL

[a] Values are means ± SD of triplicate determination.
[b] MDL: Method detection limits.

2.6. Statistical analysis

Analysis of variance (ANOVA) was done using SPSS software (Version 16.0). Significant difference at 95% confidence interval was determined using Duncan's multiple range test for comparison treatment means.

3. Results and discussion

3.1. Compositional characterization

The first step of polysaccharide characterization is measurement of its purity, which is evaluated by its chemical composition. The chemical composition of PCGE is given in Table 1. PCGE contained 71.51 ± 0.02% carbohydrate, 7.31 ± 0.04% uronic acid, 6.2 ± 0.15% moisture, 2.4 ± 0.1% protein, 2.8 ± 0.01% ash and 1.48 ± 0.13% fat. Accordingly, the majority of PCGE was carbohydrate. Carbohydrate content can be used as an indicator for the purity of gums. The amount carbohydrate in PCGE was comparable to other gum exudates such as *Prunus cerasoides* gum (73.72%) (Malsawmtluangi et al., 2014), and *Prunus armeniaca* (66.89%) (Fathi et al., 2016), and was less than *Prunus dulcis* (92.36%) (Mahfoudhi et al., 2012), *Prunus amygdalus* (98.4%) (Bouaziz et al., 2015), *Albizia procera* (85.67%) (Pachuau, Lalhlenmawia, & Mazumder, 2012), and anghouzeh gum (85.4%) (Milani, Ghanbarzadeh, & Maleki, 2012). Uronic acid content of gums reflects the relative amount of acidic polysaccharides in gum. Uronic acid content of PCGE (7.31%) was lower than those of *Prunus cerasoides* (11.05%) (Malsawmtluangi et al., 2014), *Albizia procera* (10.27%) (Pachuau et al., 2012), *Prunus armeniaca* (10.47%) (Fathi et al., 2016), and anghouzeh gum (17.8%) (Milani et al., 2012). As a result, PCGE had lower negative charge than these gums. Monosaccharides analysis by GC-MS demonstrated the presence of L-arabinose, D-galactose, xylose, mannose and rhamnose in molar percentages of 31.31%, 24.04%, 21.31%, 17.63% and 5.69%, respectively (Table 1). The monosaccharide constituent of PCGE was in consistent with monosaccharide composition of other *Prunus* gums (Malsawmtluangi et al., 2014; Nussinovitch, 2009), with small quantitative differences. There was some variability in monosaccharide composition of PCGE evidenced here compared to those reported in previous study by Amarioarei, Spiridon, Lungu, and Bercea (2011). This variability could be related to the different growing conditions, purification technique and also different method of monosaccharide analysis.

Protein content can be utilized as a useful parameter for distinguishing gums from different species (Pachuau et al., 2012). Additionally, it has also been reported that some properties of a gum including film formation ability, emulsifying and stabilizing properties arise from the protein fraction (Mhinzi, 2002; Pachuau

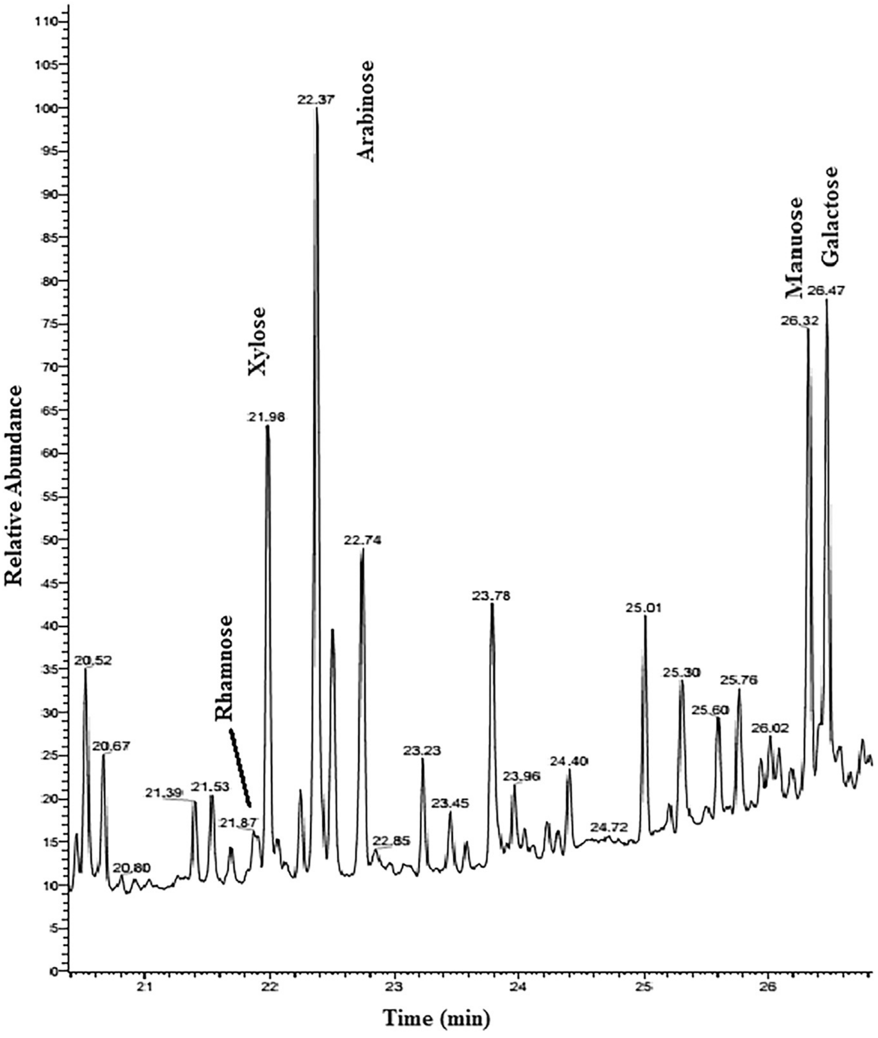

Fig. 1. Chromatogram of PCGE and its sugar composition as analyzed by GC-MS.

et al., 2012; Randall, Phillips, & Williams, 1988). Comparatively, the protein level of PCGE (2.4%) was approximately similar to the values reported for *Prunus cerasoides* gum (2.33%) (Malsawmtluangi et al., 2014), *Prunus dulcis* gum (2.45%) (Mahfoudhi et al., 2012), *Prunus armeniaca* gum (2.91%) (Fathi et al., 2016), and Arabic gum (2.18%) (Yebeyen, Lemenih, & Feleke, 2009), but was less than those reported for *Albizia procera* gum (7.34%) (Pachuau et al., 2012).

PCGA had similar fat content (1.48%) to *Prunus armeniaca* gum (1.59%) (Fathi et al., 2016), but the fat content was high compared to the value reported for *Prunus dulcis* gum (0.85%) (Mahfoudhi et al., 2012).

Ash content of PCGE samples (2.8%) was high in comparison to *Prunus cerasoides* gum (2.55%) (Malsawmtluangi et al., 2014), *Prunus dulcis* gum (2.30%) (Mahfoudhi et al., 2012), Zedo gum (1.675%) (Fadavi, Mohammadifar, Zargarran, Mortazavian, & Komeili, 2014), and lower than the data reported for *Albizia procera* gum (4.1%) (Pachuau et al., 2012), *Prunus armeniaca* gum (4%) (Fathi et al., 2016) and Arabic gum (3.56%) (Yebeyen et al., 2009).

Exudates gums are polysaccharides including various metal ions and neutralized cations (Pachuau et al., 2012). These ions are suggested to influence viscosity and gelling properties of exudates gums. For example, it is reported that viscosity and gel formation properties in some tree gum exudates such as *Khaya grandifoliola* profoundly affected by calcium ions (Aslam, Pass, & Phillips, 1978). As it can be observed in Table 1, PCGE has a considerable level of minerals. In comparison, the PCGE had higher iron (Fe), sodium (Na), phosphorus (P), potassium (K) and zinc (Zn) contents than those of Arabic gum (Yebeyen et al., 2009), Zedu gum (Fadavi et al., 2014), and *Prunus dulcis* gum (Mahfoudhi et al., 2012), but mineral contents of PCGE is approximately similar to *Prunus armeniaca* gum (Fathi et al., 2016) (see Fig. 1).

3.2. FTIR analysis

FTIR spectrum is typically used to investigate the presence of major functional groups in the structure of natural products. Fig. 2 illustrates the FTIR spectrum of PCGE sample. As it can be seen, the FTIR spectrum shows all typical bonds and peaks characteristic of polysaccharides. The area between 800 and 1200 cm^{-1} is introduced as “finger print” area for carbohydrates. This region can be used for identification of the major chemical groups in polysaccharides. A shoulder at 848.74 and 897.78 cm^{-1} is an evidence for the presence of β- and α-linkage in the structure of polymer (Percival & Percival, 1962).

The absorption at 1033.66 cm^{-1} and 1236 cm^{-1}may be associated with the presence of uronic acid and *o*-acetyl groups, respectively (Pachuau et al., 2012). Additionally, 1033.66 cm^{-1} wavenumber could be caused by C—N (Fathi et al., 2016). Thus, it could be concluded that PCGE is a polymer containing proteins which was also confirmed by chemical analysis. Peak at 1614 cm^{-1} is due to the COO groups and valence vibration, whereas the absorption at 1426 refers to $-CH_3$ and $-CH_2$ bonding vibration. Carboxyl groups can act as binding sites for ions such as calcium which these bonds have a considerable influence on the gel forming and viscosity properties (Razavi, Cui, Guo, & Ding, 2014). A small peak at 2930.42 cm^{-1} is related to C—H stretching, symmetric and asymmetric of the free sugar and also can be associated with the doubles overlapping with O—H (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). A broad range of wavenumbers between 3500 and 3000 cm^{-1} is related to O—H stretching vibration and demonstrate several features consisting of O—H groups stretching bonds which occur in samples in the vapor phase and linked to hydroxyl bonds of carboxylic acid (Silverstein, Webster, Kiemle, & Bryce, 2014).

3.3. NMR spectroscopy

The NMR analysis of PCGE is in consistent with GC-MS and FTIR analysis described above. ^{13}C and ^{1}H spectra of PCGE gum are shown in Fig. 3. ^{13}C spectrum showed the major peaks including: the range between 60 and 70 ppm which is corresponded to $C_{6,}$ and 70—81 ppm which is related to C_2, $C_{3,}$ and C_5 with overlapped signals. In addition, the peaks observed in the range of 81—93 ppm and 102—108 ppm is associated with C_4 and C_1 anomeric carbon, respectively. C_1 signal at δ 107.6—109.7 is due to the presence of α-L-Ara*f* units in the molecule (Delgobo, Gorin, Tischer, & Iacomini, 1999) and the main one at δ 103.2 corresponded to *β*-D-Gal*p* units (Tischer, Gorin, & Iacomini, 2002). Those at δ 101.5 (101.2—101.4) is related to C_1 of *β*-L-Ara*p* (Bock, Pedersen, & Pedersen, 1984; Simas et al., 2008). These results demonstrated the presence of arabinogalactan like structure in PCGE. The group of signals at δ 80.4—84.1 could be attributed to C_2 to C_4 of α-L-Ara*f* units (Gorin & Mazurek,

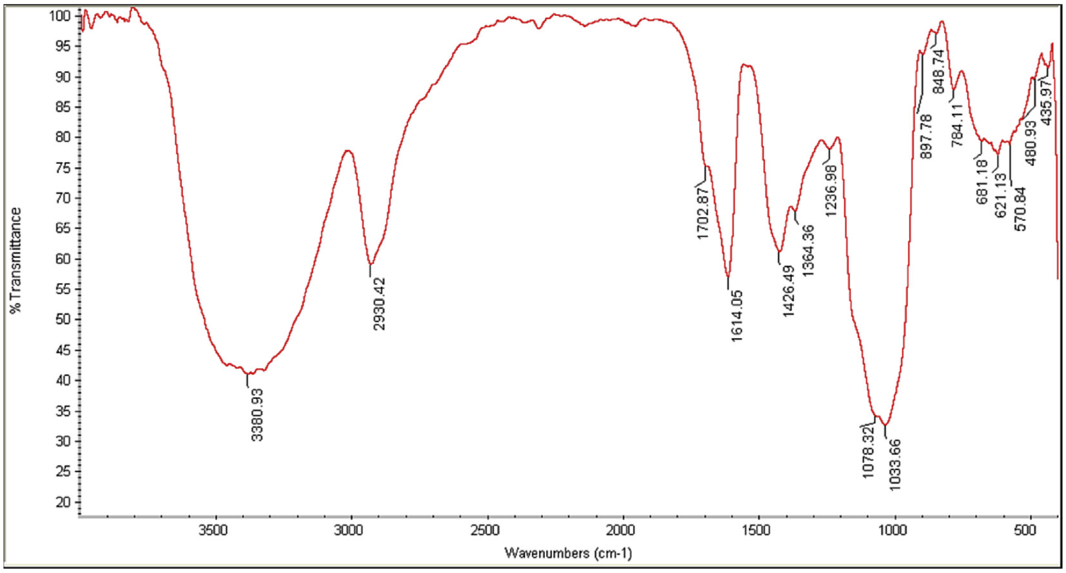

Fig. 2. FTIR spectra of PCGE.

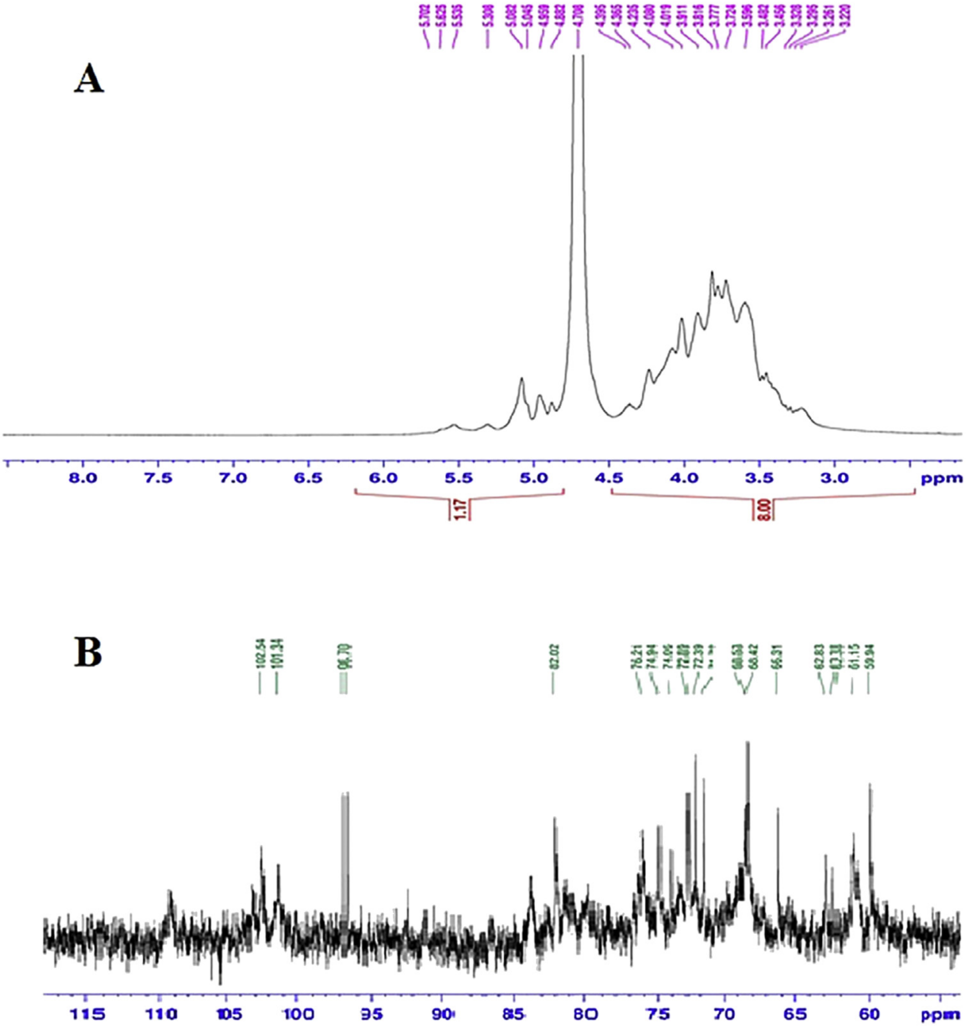

Fig. 3. ^{13}C (A) and ^{1}H (B) spectra of PCGE.

1975). The signal at δ 68.53 refers to substituted C_6 with β-D-Gal*p* units (Delgobo et al., 1999). The signal at δ 61.15 could be due to the presence of non-substituted β-D-Gal*p* units, although this signal can also be related to C_5 of α-L-Ara*f*units (Delgobo et al., 1999), however, the main signal at δ 62.83 could be associated to C-5 of β-L-Ara*p* units and non reducing end-units from α-D–Xyl *p* (Delgobo et al., 1999; Gorin & Mazurek, 1975). PCGE gum contained a signal at 60.2 ppm (with a corresponding ^{1}H at 3.48 ppm) which is related to O-Methyl group (–O–CH_3) (Simas et al., 2004). The non anomeric protons (H2-H6) were assigned between δ 3.3 and 4.6. The ^{1}H resonance at 5.08 ppm is caused by the presence of L-Ara*f* residue (Simas-Tosin et al., 2009).

3.4. Molecular weight parameters

The elution profile of PCGE in gel permeation chromatography is shown in Fig. 4. PCGE had two small peaks followed by a major peak indicating that PCGE had three fractions with different molecular weight. Weight average molecular weight (Mw) of PCGE (1.119×10^7 g/mol) was more than those reported for *Prunus armeniaca* (5.69×10^5 g/mol) (Fathi et al., 2016), *Prunus cerasoides* (5.5×10^5 g/mol) (Malsawmtluangi et al., 2014), *Prunus persica* (5.61×10^6 g/mol) (Simas-Tosin et al., 2009), summer *Prunus avium* (2.99×10^5 g/mol), winter *Prunus domestica* (1.78×10^6 g/mol), summer *Prunus cerasus* (1.3×10^6 g/mol), winter *Prunus cerasus* (1.19×10^6 g/mol) (Amarioarei, Lungu, & Ciovica, 2011), and was less than data reported for gum ghatti (8.94×10^7 g/mol) (Kaur, Singh, & Singh, 2009) (Table 2). Various factors affect the physicochemical properties of gums such as source, age of tree, time of exudation, the purification method, the contamination of these hydrocolloids with other natural compounds and growing conditions. Therefore, the main reason for the different molecular weight of PCGE is probably due to exudation date and different growing conditions.

Polysaccharides with high molecular weight do not have much of a tendency to be adsorbed at air–water interface, but they can profoundly improve the stability of protein foams by acting as thickening or gelling agents (Martinez, Baeza, Millan, & Pilosof, 2005). Therefore, it is expectable that PCGE can be utilized as a stabilizer for protein foams.

Number average molecular weight (Mn) of PCGE was found to be 4.153×10^6 g/mol which is more when compared to *Prunus armeniaca* (4.33×10^5 g/mol) (Fathi et al., 2016), anghouzeh (1.3303×10^5 g/mol) and arabic gum (2.5947×10^5 g/mol) (Milani et al., 2012).

Polydispersity index (PDI = Mw/Mn) indicates the homogeneity and molecular weight distribution of hydrocolloids. The PDI value for PCGE was 2.69 which is higher than the value reported for

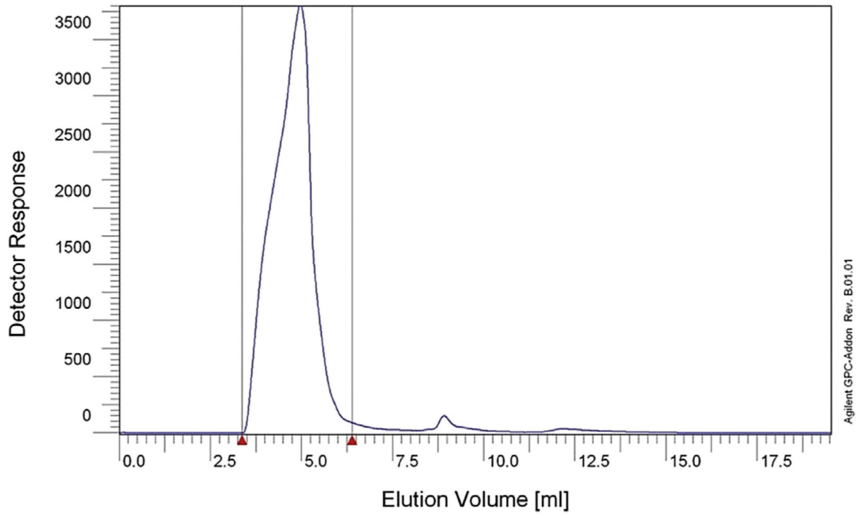

Fig. 4. The elution profile of PCGE in GPC, using refractive index detector.

Prunus armeniaca (1.31) (Fathi et al., 2016), anghouzeh (1.0256) and Arabi gum (1.2652) (Milani et al., 2012), indicating that PCGE was less uniform than *Prunus armeniaca,* Arabi gum and anghouzeh.

3.5. *Intrinsic viscosity of PCGE*

Determination coefficient values for all models are tabulated in Table 3. Obviously, Kraemer model provided better determination coefficient (R^2) among models used for determining the intrinsic viscosity of PCGE.

Intrinsic viscosity of gums depends on molecular structure, solvent quality and weight of macromolecules (Cerqueira et al., 2009). The intrinsic viscosity value for PCGE was obtained to be 3.7 dl/g based on the Kraemer equation, which was high compared to the value reported for *Prunus dulcis* (1.73 dl/g in 0.5 M KCl at 20 °C), ghatti gum (0.97 dl/g) (Kaur et al., 2009), Anghouzeh (0.213 dl/g), (Milani et al., 2012), Albizia gum (0.23 dl/g) (De Paula, Santana, & Rodrigues, 2001), Arabic gum (0.6 dl/g) (Mothe & Rao, 1999), Cashew gum (0.1 dl/g) (Mothe & Rao, 1999), and was comparable the value reported for *Prunus armeniaca* L (3.438 dl/g) (Fathi et al., 2016).

The physical meaning of shape factor can be expressed as follow: (a) $\nu = 2.5$ shows spherical shape and (b) $\nu > 2.5$ indicate the ellipsoidal particles. Shape factor value for PCGE was estimated to be 3.23 and therefore, PCGE has an ellipsoidal-like configuration.

Since the berry number for dilute PCGE solution was 0.97, the molecular conformation of this gum is probably rod-like (Lai & Chiang, 2002; Lapasin, 2012; Morris, Cutler, Ross-Murphy, Rees, & Price, 1981).

The coil radius (18.7 nm) and volume (27377 nm^3) for PCGE in deionized water (at 25 °C) were more than those reported for *Prunus armeniaca* (6.77 nm and 1299.07 nm^3) (Fathi et al., 2016), sage seed gum (6.23 nm and 1012.35 nm^3) (Yousefi, Razavi, & Aghdam, 2014), and the values for *Alyssum homolocarpum* seed gum (11.10 nm and 5732.50 nm^3) (Hesarinejad, Razavi, & Koocheki, 2015).

3.6. *Steady shear properties*

Fig. 5 illustrates the viscosity-shear rate profile of PCGE solutions at 25 °C as a function of gum concentration. By increasing shear rate, the viscosity of gum solutions decreased which demonstrates the pseudoplastic behaviour of PCGE solutions. This behaviour is expectable for polysaccharides solutions due to their polymeric structure and high molecular weight (Rao, Suresh, & Suraishkumar, 2003). As shear rate increases, the randomly positioned chains of polymer molecules become aligned in the direction of the flow, produce solutions with less viscosity, and cause lower interaction across adjacent polymer chains (Koocheki,

Table 3
Intrinsic viscosity determination of PCGE based on different equations[a].

Model	[η] (dl/gr)	R^2
Huggins	4.04 ± 0.08	0.92
Kraemer	3.70 ± 0.04	0.95
Tanglertpaibul and Rao	2.75 ± 0.05	0.83
Higiro 1	2.30 ± 0.04	0.60
Higiro2	4.71 ± 0.08	0.65

[a] Values are means ± SD of triplicate determination.

Table 2
Weight average molecular weight of some gum exudates.

Prunus species	Molecular weight (g/mol)	References
Prunus cerasus	1.119×10^7	Current study
Prunus persica	5.61×10^6	Simas-Tosin et al. (2009)
Prunus persica	4.60×10^6	Qian, Cui, Wang, Wang, and Zhou (2011)
Prunus domestica (winter)	1.78×10^6	Amarioarei, Lungu et al. (2011)
Prunus cerasus (summer)	1.3×10^6	Amarioarei, Lungu et al. (2011)
Prunus cerasus (winter)	1.19×10^6	Amarioarei, Lungu et al. (2011)
Prunus avium (summer)	2.99×10^5	Amarioarei, Lungu et al. (2011)
Prunus cerasoides	5.5×10^5	Malsawtluangi et al. (2014)
Prunus armeniaca	5.69×10^5	Fathi et al. (2016)

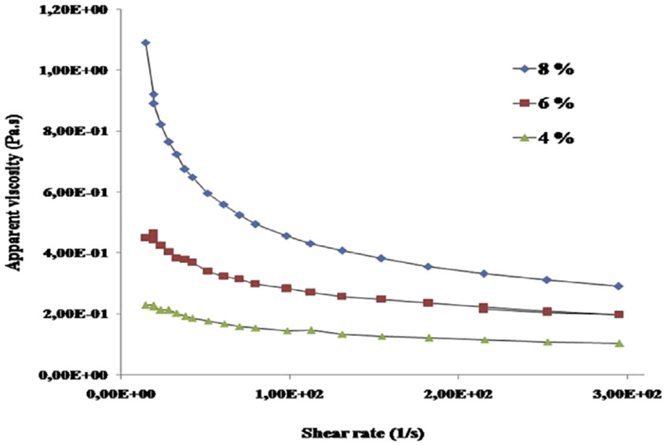

Fig. 5. The effect of different concentrations on apparent viscosity of PCGE.

Taherian, & Bostan, 2013). At high shear rates, the decrease in viscosity may be associated with a reduction in number of chain entanglements (Nehdi, 2011). Over the shear rate range between 10 and 100 s^{-1}, solutions with more concentration showed higher pronounced shear thinning behaviour.

The consistency coefficient (k) and flow behaviour index (n) values corresponding to the upward and downward curves were determined by fitting the shear stress-shear rate profile data based on the power law model. The results for 4, 6 and 8% gum solutions are summarized in Table 4.

The determination coefficients were close to 1 which showed the appropriateness of power law model for describing the rheological behaviour of the PCGE solutions. All solutions showed a non-Newtonian shear thinning behaviour ($n < 1$). Shear thinning fluids are appropriate to be pumped easily. Thus, PCGE could be used as a suitable fluid for all processes including pumping fluid. Moreover, it is clear that an increase in PCGE concentration led to an increase of the pseudoplasticity, which can be distinguished by decreasing in the value of flow behaviour index (n). However, it caused an increase in the consistency coefficient (an indicator of the viscous nature of gum). This increasing trend could be attributed to the higher solid contents which generally lead to an increase in the viscosity which is the consequence of molecular movement and interfacial film formation (Maskan & Göğüş, 2000). From a sensory point of view, a high flow behaviour index value for gum solutions can create a slimy feel mouth which is undesirable (Szczesniak & Farkas, 1962). Therefore, to obtain a good mouth feel, we should use a gum system with a rather high concentration.

There were differences in the consistency coefficient and flow behaviour index values between the upward and downward curves which could be attributed to time dependent behaviour of PCGE solutions (Fig. 6). These results are in agreement with those reported for *Alyssum homolocarpum* seed gum (Koocheki & Razavi, 2009), *Lepidium perfolatium* (Koocheki et al., 2013) and *Salvia hispanica* L. mucilage (Capitani et al., 2015).

Compared to downward curves, upward curves had higher k values and lower n values. Accordingly, PCGE solutions showed slightly a thixotropic behaviour. Therefore, it is clear that at a constant shear rate, an increase in shearing time led to a decrease in apparent viscosity due to the progressive structural breakdown of PCGE solutions (Abu-Jdayil & Mohameed, 2004).

3.7. *Oscillatory measurement*

The frequency sweep experiments were done at the constant strain of 1% where was in the linear viscoelastic region. This test can be utilized to characterize and classify dispersions. There are four classifications which are dilute solution, concentrated solution, a weak gel and strong gels (Steffe, 1996). The mechanical spectra of PCGE as a function of gum concentration in deionized water at 25 °C is given in Fig 7. In the range of frequency tested, gum solutions demonstrated typical viscoelastic characteristics rather than physical gel properties. In all examined concentrations, G'' was superior to G', meaning that gum solutions had more viscous behaviour than a clear tendency to form a gel. This behaviour is in agreement with those of reported for some arabinigalactan-type polysaccharides. For example, gum Arabic (6% (w/v)) had a liquid behaviour when it was allowed to rest before experiment, which might be due to the aggregation of arabinogalactan proteins (APG) (Sanchez, Renard, Schmitt, & Lefebvre, 2002). The mechanical spectra of other arabinogalactan gums such as mesquite gum and A. *tortuosa* gum indicated a liquid-like behaviour even at highly concentrated preparations (Goycoolea, Morris, Richardson, & Bell, 1995; Muñoz et al., 2007). On the other hand, there are some gum exudates from the arabinogalactan-group which have a quite different behaviour, when compared with the PCGE solutions. Karaya (*Sterculia* sp) gum, for example, has a gel like behaviour at concentrations greater than 2% (w/v) (de Brito, Sierakowski, Reicher, Feitosa, & de Paula, 2005).

In addition, both dynamic storage modulus and loss modulus curves were almost parallel with a strong frequency dependency. This is typical behaviour for biopolymer solutions at concentrations below the critical polymer coil overlap concentration. Furthermore, with increasing PCGE concentration, G' and G'' values elevated. This increasing trend is due to the formation of complex structure at higher gum concentrations (Hesarinejad, Koocheki, & Razavi, 2014). The values of G' and G'' for 8% PCGE concentration were slightly less than that of 6%, indicating that the elastic behaviour decreased at special concentration. Similar results reported by Hesarinejad et al. (2014) and Yoo, Kim, and Yoo (2005). Further investigation is necessary to exactly explain why G' and G'' for 8% PCGE concentration were lower than that of 6%.

3.8. *Colour measurement*

The values of L*, a*, and b* in powder PCGE samples were measured to demonstrate the colour properties of PCGE. As given in Table 5, in the PCGE powders, lightness, redness, yellowness and chroma values were found to be 84.276 ± 0.219, 2.048 ± 0.043,

Table 4
Power law parameters for PCGE in different concentrations*.

Concentration (w/w) %	Upward curve			Downward curve		
	K (Pa s^n)	n	R^2	K (Pa s^n)	n	R^2
4	0.58 ± 0.08^c	0.70 ± 0.01^a	0.999	0.36 ± 0.02^c	0.77 ± 0.01^a	0.999
6	1.15 ± 0.04^b	0.67 ± 0.02^a	0.999	0.96 ± 0.01^b	0.72 ± 0.00^b	0.998
8	3.07 ± 0.06^a	0.57 ± 0.01^b	0.999	1.64 ± 0.15^a	0.69 ± 0.00^c	0.996

*Values are means ± SD of triplicate determination.

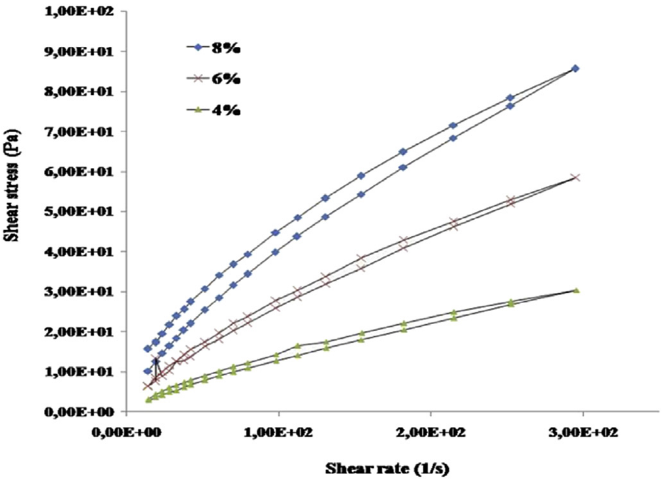

Fig. 6. Flow curves indicating thixotropy for different concentrations.

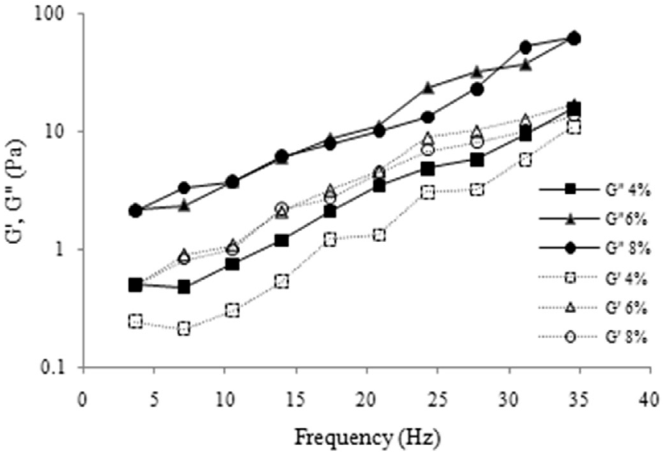

Fig. 7. Mechanical spectra of PCGE as a function of gum concentration in deionized water at 25 °C.

Table 5
The values of L*, a*, b* and C* in PCGE.

Parameter	PCGE
L^*	84.276 ± 0.219
a^*	2.048 ± 0.043
b^*	21.962 ± 0.088
C^*	22.057 ± 0.120

*Values are means ± SD of triplicate determination.

21.962 ± 0.088 and 22.057 ± 0.12, respectively. $L^*$and b^* values in PCGE were higher than those reported for *Prunus armeniaca* (Fathi et al., 2016) and extracted mesquite seed gum by acid (Estévez et al., 2004). A higher value of L*, which reflects the ratio of white to black colour means that this gum is a whitish gum rather than *Prunus armeniaca* L and mesquite seed gum. A higher value of b* parameter, which denotes the yellowness and blueness quality of the colours indicated that PCGE had more level of yellowness.

4. Conclusion

PCGE was found to be mainly composed of carbohydrate. This gum is a high molecular weight polysaccharide (1.119 × 10^7 g/mol), composed of L-arabinose, D-galactose, xylose, mannose and rhamnose. Arabinose was the most abundant monosaccharide; accounting for 31.31 molar percentage of the carbohydrate content of PCGE. FTIR analysis demonstrated the presence of carboxyl, hydroxyl, acetyl and methyl groups and glycoside bonds. ^{13}C NMR and ^1H NMR analysis demonstrated an arabinogalactan structure for this gum. Colour parameters including lightness, redness, yellowness, and chroma were found to be 84 ± 0.219, 2.048 ± 0.043, 21.962 ± 0.088 and 22.057 ± 0.12, respectively. PCGE was an ellipsoidal polymer with R_{coil} and V_{Coil} 18.7 nm and 27,377 nm, respectively. The steady shear experiments revealed that this new biopolymer can form viscous aqueous solutions with non-Newtonian shear thinning behaviour and thixotropy. Therefore, PCGE is proper for application in all processing including pumping fluids. Oscillatory shear indicated that in PCGE solutions (4, 6 and 8%), loss modulus (G″) was more than storage modulus (G′), indicating that the solutions demonstrate viscous behaviour.

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