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Influence of temperature, pH and salts on rheological properties of bitter almond gum

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Abstract

This study focuses on the rheological properties of bitter almond gum (BAG) exudate at different shear rates, concentrations, temperatures, pH, and in the presence of various salts. Rheological data fitted with the Power law model revealed that BAG solutions exhibit non-Newtonian, shear thinning behavior without thixotropic effects at all tested concentrations and temperatures. Apparent viscosity of BAG solutions increased with the increasing gum concentrations and decreased with the increasing shear rate at a specified temperature. The viscosity reached a maximum value at pH 7 and it decreased at lower and higher pH values. Salts caused a reduction in viscosity. Comparatively, $CaCl_2$ had a more pronounced effect than NaCl at a similar concentration. All treatments had significant effects on rheological parameters.

Keywords: apparent viscosity; bitter almond gum; gum exudate; rheological parameters.

Practical Application: BAG exudate behaves like most of the other gums and is potentially useful for the application in food formulations, given its low cost and availability.

1 Introduction

Exudate gums are the oldest natural gum used in different systems to produce the gels or viscous solutions and stabilize the emulsions and foams. Its production is due to a natural defense mechanism of plants against the damages caused by microorganisms, insects or mechanical injuries (Mirhosseini & Amid, 2012). They are composed mainly of polysaccharides with protein and phenolic compounds as minor components. The polysaccharides have a complex and highly branched structure, which can be classified according to their primary structures into: arabinogalactans (AG, e.g. Arabic gum), substituted glucuronomannans (e.g. Ghatti gum), substituted rhamnogalacturonans (e.g. Karaya gum) and a mixture of AG and galacturonan type regions (e.g. Tragacanth gum) (Lapasin & Pricl, 1995)

Bitter almond gum (BAG), also called *Persian*, *Angum*, *Ozo*, *Zedo* or *Zodo* gum, is a transparent exudate gum obtained from stems and branches of bitter almond tree (*Amygdalus scoparia* spach), which is native to some mountainous regions of Asia, especially Iran and India. In Iran, it is harvested in the Zagross regions and usually used for the production of glues and traditionally for wound treatment (Nussinovitch, 2010).

The BAG resembles Arabic gum in appearance and color, and is mistaken as the same. It is colorless or pale yellow to amber-brown. The gum is made of two fractions, one is soluble in water (25-30%) and the other, insoluble but water-swelled (70-75%) (Abbasi & Mohammadi, 2013). It contains about 93.1-98.4% carbohydrate, 0.19-0.21% protein, 1.4-1.7% ash and a trace amount of lipid, depending on its color. The polysaccharide moiety of the gum has a molecular weight ranging from 2590 to 4740 kDa, consisting of mostly arabinose (54.5-62.8%) and galactose (27.2-31.9%), along with very little amount of rhamnose and galacturonic acid. Hence, it has an arabinogalactan structure similar to that of Arabic gum (Fadavi et al., 2014).

The BAG is described as the salt of an acidic polysaccharide polymer (Abbasi & Mohammadi, 2013), whose structural, biological and rheological properties have not yet been fully characterized. Unfortunately, to our knowledge, there have been few studies investigating this gum, in which mostly the emulsion stability of the gum has been addressed (Jafari et al., 2013; Golkar et al., 2015). For example, this gum stabilizes D-limonen emulsions better than Arabic gum at the same concentrations (Golkar et al., 2015). On the contrary, a review of literature shows that the rheological properties of BAG are much less investigated. Because the food gums are usually exposed to different conditions during the process, the rheological properties of the gums may be affected. Therefore, this study was designed to determine the effects of different concentrations, temperatures and environmental conditions (pH and salts) on the rheological properties of the gum. The results of this study may provide further rheological information about the gum and expand its use in the food, drug and other applications.

2 Materials and methods

2.1 Materials and reagents

Crude BAG was collected from plains and mountains around Kazerun in Fars province, Iran. The gum was milled with an analytical grinder (Yellow line A10 basic, IKA-Werke,

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Germany) to get a uniform fine powder. The resulting powder was dispersed in distilled water and then, passed through a fine cloth to remove insoluble foreign matters. The filtrate was extracted with 96% v/v ethanol for 10 hours in a Soxhelet apparatus to eliminate colorants and then, dried in a vacuum oven (EHERT, Germany) at 40 °C for 12 hours (Jafari et al., 2013). All the chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2 Solutions preparation

BAG solutions (1, 2 and 3% w/v) were prepared by gradually dispersing the required amount of gum powder in 20 mM Imidazole buffer solution using a magnetic stirrer under gentle continuous stirring at 25 °C. The solutions were stored at 25 °C overnight to complete hydration prior to the experiments. The pH level of the resulting solution was 4.41 ± 0.26. The gum solutions with different concentrations of NaCl (100-300 mM) and $CaCl_2$ (5-100 mM), or different levels of pH (2, 4, 7 and 10) were separately prepared using constant gum concentration (3% w/v). The pH of solutions was adjusted with 0.1 M NaOH and HCl solutions. All sample solutions were prepared in triplicate.

2.3 Rheological measurements

The rheological properties of BAG solutions at different concentrations and temperatures were measured by a rotational programmable viscometer (Model LVDV-II Pro, Brookfield Engineering Inc., USA), equipped with a specific spindle (No. 27). The gum solution (0.5 ml) was pipetted into the sample cup and allowed to equilibrate for 20 min at desired test temperatures (5, 25 and 85 °C). Samples were then, subjected to a programmed shear rate which increased linearly from 4 to 85 s^{-1} over a 3 min period, kept constant at 85 s^{-1} for 2 min and decreased linearly to 4 s^{-1} in another 3 min period. The rheological properties as a function of pH and salt were determined just at a specified shear rate, 54.4 s^{-1} and temperature, 25 °C.

2.4 Statistical analysis and calculations

The rheological measurements of BAG solutions were determined by fitting shear stress against shear rate data to the Power law model according to following Equation 1:

$$\tau = k\dot{\gamma}^n \quad (1)$$

Where, τ is the shear stress (mPa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency index ($mPa.s^n$), and the exponent n is the flow behavior index. The linear regression models were also used to minimize the difference between the model and data points through CurveExpert software package (Version 1.40, USA), at a significant probability level of 95%. One-way ANOVA and Duncan test were applied to establish any significant difference among the mean values of rheological parameters at $P < 0.05$. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 Chemical composition of BAG

Chemical analysis of BAG showed that the gum is rich in carbohydrate, 97.33% db, with no lipid content. The protein and ash contents of the gum were 0.52 and 2.15% db, respectively. These results were within the range of values reported by Fadavi et al. (2014) and Golkar et al. (2015), and were similar to that of Tragacanth (Gorji et al., 2014). The carbohydrate and ash contents were relatively similar, while the protein content was relatively low (0.52 vs 2.1-2.5%), in comparison to other exudate gums such as Mesquite (Orozco-Villafuerte et al., 2003) and Arabic gum (Gashua et al., 2015),.

3.2 Concentration and temperature dependency

The steady flow curves of BAG solutions (1-3% w/v) at shear rate range of 4.08-85 s^{-1} and 25 °C are shown in Figure 1a. In all samples, the gum solutions showed a non-Newtonian, shear thinning behavior, which was more pronounced with the increasing

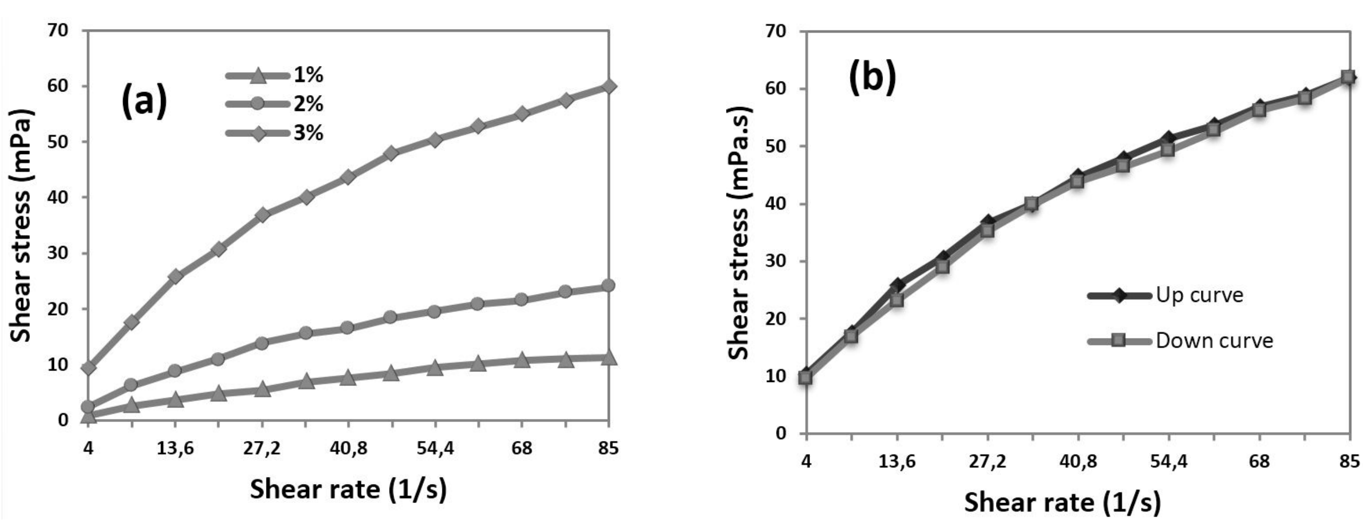

Figure 1. Steady shear curve (**a** - 1-3% w/v) and thixotropic behaviour (**b** - 3% w/v) of BAG solutions at 25 °C.

gum concentration and shear rates. Figure 1b shows that gum solution (3% w/v) at shear rates used (from 4 to 85 s^{-1}) was not a thixotropic fluid. As it is evident, the gum solution recovered its almost original shear stress and viscosity after returning at the same rate to 4 s^{-1} on standing for 10 min. This reversibility is attributed to structural rebuild up of the broken gum network at rest, which makes the BAG be a suitable hydrocolloid in sauces and salad makings, allowing the food to display a certain consistency during the processing and storage (Lapasin & Pricl, 1995). These findings are in agreement with those found for other gum solutions such as Guar gum (Torres et al., 2014) and Tara gum (Wu et al., 2015).

Figure 2 shows the viscosity dependence of the 3% gum solution on changing the shear rates (from 4.08 to 85 s^{-1}) and temperatures (from 5 to 85 °C). For all samples, the apparent viscosity of the gum solution decreased with the shear rate increasing at a specified temperature. Additionally, the viscosity was affected by changing temperature and shear rate simultaneously and sharply decreased at low temperatures used (5 and 25 °C), and the lower shear rates of 40.8 s^{-1}. With increasing temperature, the energy required for molecular mobility of the gum is provided. Therefore, the resistance of fluid to flow is decreased, leading to decrease in viscosity during heating (Milani et al., 2012; Abbastabar et al., 2015). This behavior may be due to thermal degradation of high molecular weight gums into gums with varying molecular weights during heating (Abbes et al., 2015).

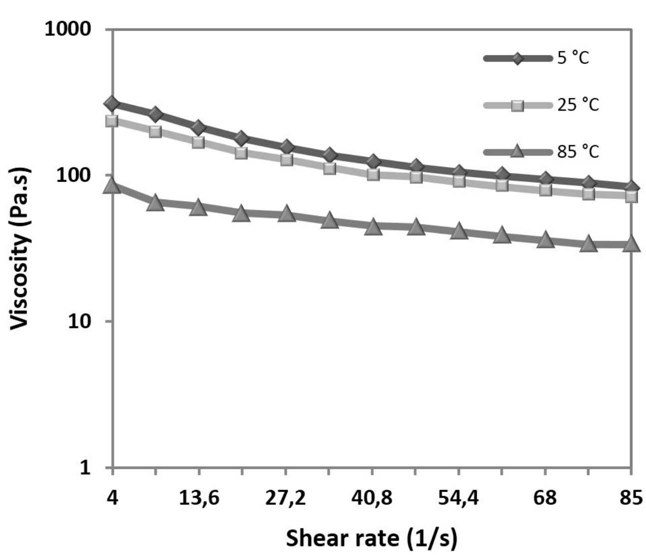

Figure 2. Effect of different temperatures and shear rates on apparent viscosity of 3% BAG solution.

Thermal degradation rate of the gums is often linked to their activation energy and molecular weight, and increases with decrease of gum molecular weight. Kok et al. (1999) showed that some gums such as Guar gum (GG) in spite of higher molecular weights, but relatively lower activation energy, is thermally more susceptible than Locust bean gum (LBG). They suggested that LBG possesses a greater ability to associate in solution, which can protect the gum against thermal degradation, as compared with GG (Stokke et al., 1992). The rate of hydration by gum molecules can also affect the flow of BAG solution (Wang et al., 2015). Mudgil et al. (2014) stated that, the Guar gum solutions subjected to a high temperature hydrate much faster than those exposed to a low temperature, but they display a lower final viscosity as a result of loss of hydration.

The rheological data fitted on the basis of Power law model are summarized in Table 1. The coefficient of determination (R^2) for all tested BAG solutions were 0.98, indicating that the Power law model is an appropriate model for describing the flow behavior of BAG solutions. As presented in Table 1, a shear thinning behavior (n less than 1) is observed for all BAG solutions at different concentrations and temperatures. This behavior is reported for other exudate gums such as kondagogu (Janaki. & Sashidhar, 1998), Mesquite (Orozco-Villafuerte et al., 2003), Tragacanth (Balaghi et al., 2011), Ghatti (Kang et al., 2011) and Arabic gum (Gashua et al., 2015). Shear thinning behavior is mainly due to the breakdown of structural units of the gum and rearrangement of its chains in direction of hydrodynamic forces generated during shear (Vardhanabhuti & Ikeda, 2006). This behavior can give rise to less interaction among adjacent gum chains, leading to decrease of viscosity.

The increase of gum concentration resulted in k value increasing at a specific temperature. The k value was also affected by temperature at a constant concentration, so that increasing temperature from 5 to 85 °C led to decrease of k value. The k reflects the values of the viscosity, so the main reasons for k value variations are the same as those mentioned above for viscosity variation. Generally, the n and k values are changed conversely. Flow behavior index for all gum solutions were in the range of 0.51-0.95 and tended to be close to Newtonian behavior, as temperature increased. The temperature and gum concentration had a remarkable inverse effect on n value. For all cases, n values increased with increase of gum concentration and decrease of temperature, simultaneously. With increasing temperature, the average kinetic energy per molecule increases, which can lead to increase in molecular mobility and flow behavior index (Kok et al., 1999; Abbes et al., 2015).

Table 1. Effect of different BAG concentrations and temperatures on Power law parameters.

Gum (%w/v)	k (mPa.s^n)			n		
	5 °C	25 °C	85 °C	5 °C	25 °C	85 °C
1	3.4 ± 0.2^{Ca*}	2.1 ± 0.5^{Cb}	0.3 ± 0.1^{Cc}	0.81 ± 0.00^{Ac}	0.88 ± 0.02^{Ab}	0.95 ± 0.07^{Aa}
2	18.1 ± 0. 2^{Ba}	11.4 ± 0.4^{Bb}	2.9 ± 0.3^{Bc}	0.61 ± 0.02^{Bc}	0.67 ± 0.01^{Bb}	0.84 ± 0.00^{Ba}
3	77.6 ± 0.9^{Aa}	60.5 ± 0.7^{Ab}	18.6 ± 0.4^{Ac}	0.51 ± 0.03^{Cb}	0.52 ± 0.01^{Cb}	0.62 ± 0.04^{Ca}

*Different small or capital letters show significant difference in the same row or column, respectively ($p \leq 0.05$).

3.3 pH dependency

The effects of different pH levels (2, 4, 7 or 10) on the viscosity of BAG solution (3% w/v) at a specific shear rate (54.4 s^{-1}) and 25 °C are displayed in Figure 3. The results showed that changing pH has a significant effect on the rheological parameters at pH values studied (Chen et al., 2006; Abbastabar et al., 2015). The viscosity reached a maximum value at pH 7 and it decreased at lower and higher pH values. The lowest viscosity was obtained at pH 2. The apparent viscosity significantly increased over the pH values from 2 to 7 and then decreased with increasing pH from 7 to 10.

The k and n indices were significantly affected by changing pH value. Generally, these are changed inversely. As evident in Table 2, the maximum k was obtained at neutral pH, whereas the n value tends to be the lowest at this pH values. The k value significantly changed with varying pH values, while the n value changed under alkali condition greatly, but no significant change was seen at acidic condition. This is consistent with the results obtained for flaxseed gum, xanthan gum (Renaud et al., 2005; Qian et al., 2012), Konjac gum (Jian et al., 2015) and cashew gum (Porto et al., 2015).

The natural pH of BAG solution found to be 4.41 ± 0.26, so the gum is an anionic polysaccharide containing a great number of negative charge-bearing groups such as carboxyl groups, which may undergo different degrees of ionization with changing pH value, leading to changes in viscosity and rheological properties (Lapasin & Pricl, 1995). Also, the pH-dependence of viscosity may be due to change in gum conformation. At low pH, polysaccharide chains tend to appear in coil state with acid groups in free acid form. With increasing pH, acid groups of coils are gradually ionized and the coils are expanded due to increase in electrostatic repulsion between functional groups, leading to more intermolecular interactions among the coils and consequent higher viscosity of solution (Feng et al., 2007).

The maximum viscosity was obtained around the pH 7.0 for BAG solution, where the shape of hydrocolloids chains is close to rod conformational state (Achi & Okolo, 2004). This condition usually appears at pH values lower than 9, where acid groups are ionized and electrostatic repulsion reaches a maximum and consequently, tends to keep the molecules in an extended form, leading to a high viscous solution and higher k values (Medina-Torres & La Fuente, 2000; Coupland, 2013) The decrease of viscosity from pH 7 to 10 may be explained by the neutralization effect of added alkali on the negative charges of the gum, which reduces the hydrodynamic volume of the gum and consequent viscosity (Chen et al., 2006; Porto et al., 2015) and also gum depolymerization under alkali condition, proposed by Achi & Okolo (2004).

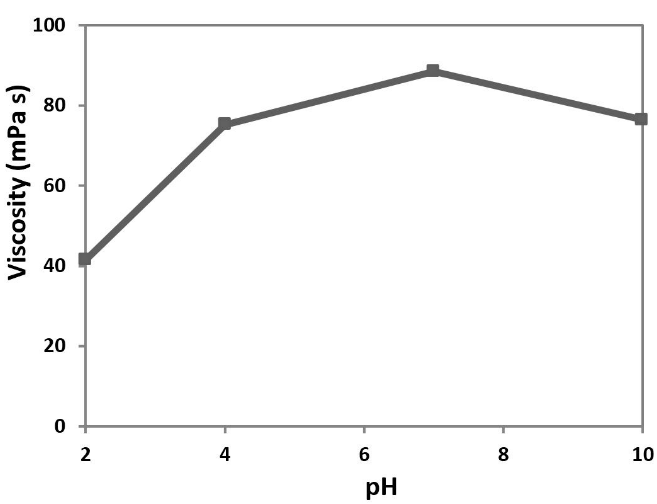

Figure 3. Effect of different levels of pH on apparent viscosity of 3% gum solution at shear rate 54.4 S^{-1} and 25° C.

Table 2. Effect of different levels of pH on Power law parameters of 3% BAG solutions at shear rate 54.4 s^{-1} and 25 °C.

pH	2	4	7	10
k ($mPa.s^n$)	394 ± 1.1^{a*}	467 ± 1.1^{b}	605 ± 3.5^{c}	410 ± 7.8^{a}
n	0.55 ± 0.01^{ab}	0.54 ± 0.00^{ab}	0.52 ± 0.01^{a}	0.58 ± 0.04^{b}

*Different small letters show significant difference in the same row (p ≤ 0.05).

3.4 Salts dependency

Food gums mostly serve as a polyelectrolyte and react with salts contained in food (Salehi et al., 2014). These interactions may cause changes in rheological characters and biological properties of the gums. In Figure 4 the effect of NaCl (100-500 mM) and $CaCl_2$ (5-100 mM) on the apparent viscosity of BAG solution (3% w/v) with changing shear rate (from 4 to 85 s^{-1}) are displayed, respectively. It was observed that gum solutions with lower salt concentration exhibited greater viscosity. In addition, viscosity declined as shear rate increased with rising salt concentration, especially at the lower shear rates of 40.8 s^{-1}. However, the gum solution still remained shear thinning, regardless of salt type and concentration. Such a behavior has been observed for many gum solutions (Salehi & Kashaninejad, 2015; Wu et al., 2015). The BAG is an anionic gum, which may be influenced by the introduced cations. The effects of salts on viscosity and rheological characters of the gum solution are presumably due to changing gum molecular conformation. Gum solution containing no salt exhibits high viscosity due to highly expanded molecules in the medium. At lower salt concentrations, the electrostatic screening effect of salts around the gum markedly limits the gum extension and declines the viscosity of gum solutions dramatically. With increasing salt concentration, the negatively charged residues on gum chains are exposed to the cations of the salts and neutralize, thus the gum conformation collapses to a more compact coil. This leads to a decrease in more hydrodynamic volume of the gum and electrostatic repulsion among gum chains and consequently results in further viscosity reduction (Carrington et al., 1996).

The effects of salts (NaCl and $CaCl_2$) on the apparent viscosity and rheological Power law parameters of BAG solution (3% w/v) at a specific shear rate (54.4 s^{-1}) and 25 °C are summarized in Table 3. The μ_a clearly decreased from 0.09 to 0.02 Pa.s, with increasing each of the salts. Comparatively, $CaCl_2$ had a more pronounced effect than that observed for NaCl at similar concentration of the salt used. For example, inclusion of 100 mM $CaCl_2$ to 3% w/v gum solution decreased μ_a value up to about

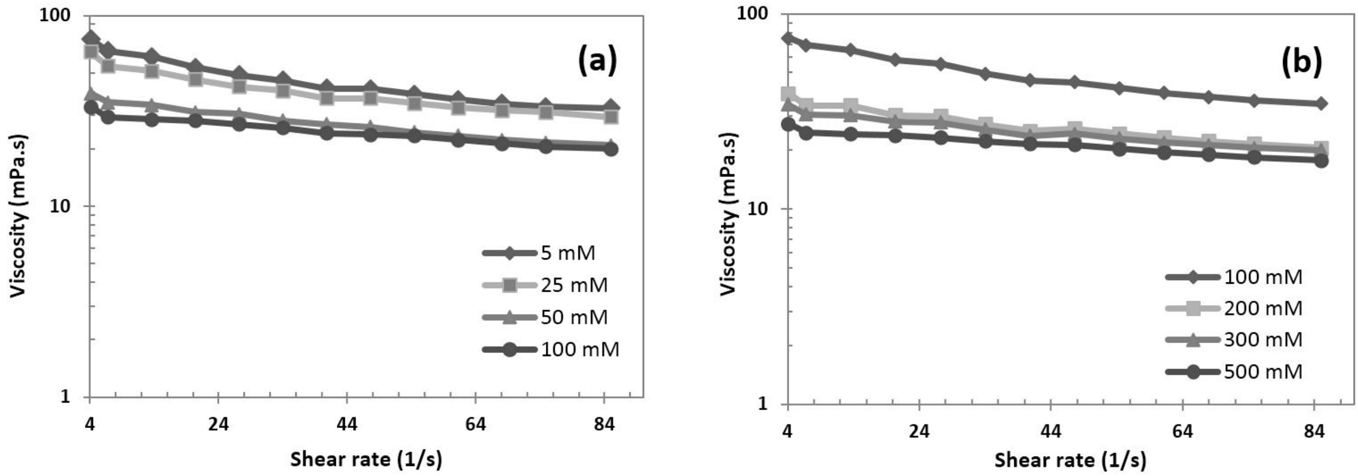

Figure 4. Effect of different concentrations of NaCl (**a**) and $CaCl_2$ (**b**) on apparent viscosity of 3% BAG.

Table 3. Effect of different concentrations of NaCl and $CaCl_2$ on Power law parameters and apparent viscosity for upward curves of 3% BAG solution at shear rate 54.4 s^{-1} and 25 °C.

Salt (mM)	Control	NaCl (mM)				$CaCl_2$ (mM)			
	0	100	200	300	500	5	25	50	100
k ($mPa.s^n$)	$605 \pm 34^{a*}$	161 ± 3.4^{b}	63 ± 6.9^{c}	53 ± 1.8^{c}	43 ± 5.9^{c}	139 ± 7.5^{b}	108 ± 0.5^{b}	60 ± 4.2^{c}	53 ± 0.6^{c}
n	0.52 ± 0.05^{d}	0.66 ± 0.01^{c}	0.76 ± 0.02^{b}	0.78 ± 0.00^{ab}	0.82 ± 0.03^{a}	0.68 ± 0.01^{c}	0.71 ± 0.00^{b}	0.77 ± 0.01^{ab}	0.79 ± 0.00^{ab}
Viscosity (mPa.s)	90 ± 1.5^{a}	41 ± 0.2^{b}	24 ± 0.5^{c}	23 ± 0.3^{c}	20 ± 0.4^{c}	38 ± 0.1^{b}	34 ± 0.4^{bc}	24 ± 0.3^{c}	22 ± 0.1^{c}

*Different small letters show significant difference in the same row ($p \leq 0.05$).

80%, while it dropped to about 55% in the presence of 100 mM NaCl. Oliveira et al. (2001) studied rheological properties of E. contortisilliquum gum in the presence of Na^+, Ca^{2+} and Al^{3+} at a same ionic strength (I=1) and concluded that cation affinity of gum molecule is dependent on the charge to ionic radius ratio of counter ions. Lower value of charge to ionic radius ratio induces lower chain contraction for gum molecules in solution. Hence, gum solutions (at infinite dilution) containing Na^+ or Al^{3+} produce the highest or least viscosity, respectively, while Ca^{2+} exerts an intermediate chain contraction and viscosity. Medina-Torres & La Fuente (2000) studied rheological properties of *Opuntia ficus indica* mucilage gum and showed that Ca^{2+} and Mg^{2+} ions have a more significant effect on viscosity decreasing, compared to Na^+ and K^+ ions. This order of interaction has been observed between counter ions and gums such as Albizia lebbeck gum (Paula et al., 2001), Balengo gum (Amini & Razavi, 2012) and karaya (Raizadayb et al., 2015).

Variations in μ_a and k values were only significant up to 25 mM $CaCl_2$ and 100 mM NaCl, while the n value significantly increased with salt increasing, regardless of salt type. The n value increased to 0.79 and 0.82 with increasing concentration of NaCl (0-500 mM) and $CaCl_2$ (0-100 mM), respectively (Figure 4 and Table 3). Lai & Chiang (2002) studied rheological properties of hessian-tsao leaf gum and concluded that gum solution with n values less than unit tend to be more in rod-like conformation than random coil one. Higiro et al. (2007) showed that increase in n values of xanthan-locust bean gum mixture solution with increasing salt concentration is likely the result of change in molecular conformation of gum from rod-like to random coil one.

4 Conclusions

The Power law model properly described the flow behavior of BAG solutions. The temperature, pH variation and adding salts significantly affected rheological properties of the gum. Apparent viscosity of the gum increased with the increasing gum concentrations. The maximum viscosity found to be at pH 7, whereas it was the least under acidic or alkali conditions. Salts (NaCl and $CaCl_2$) caused a reduction in viscosity. Comparatively, $CaCl_2$ had a more pronounced effect than NaCl at a similar concentration. The results also showed that BAG exudate is potentially useful for the application in food due to low cost and its availability. Further studies are needed to fully investigate the effects of other commonly used ingredients in food, drug and other systems on this gum.

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