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Rheological study of galactomannan depolymerisation at elevated temperatures: Effect of varying pH and addition of antioxidants

M. Samil Kök*

Abant İzzet Baysal University, Faculty of Engineering-Architecture, Department of Food Engineering, 14280 Bolu, Turkey

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ABSTRACT

Guar gum (GG) and locust bean gum (LBG) are neutral and should not be expected to change viscosity at different pH values. However, heat treatment at 100 °C within the range of pH values 5.0–9.0 did show significant differences in viscosity. Lowest viscosity and therefore probable maximum degradation was found at pH 7.0 which is indicative of free radical attack being optimum at this pH.

Antioxidants, sodium sulphite (SS) and propyl gallate (PG), added at 1000 and 500 ppm, respectively, provide both gums with stability when subjected to temperature treatments up to 121 °C. This also supports the proposal that it is the action of free radicals at higher temperatures that causes thermal degradation. LBG shows a better conservation of viscosity than GG, on addition of antioxidants, suggesting that the protective effect of its intermolecular associations is further enhanced by the antioxidants.

1. Introduction

Galactomannans, also known as "seed gums", are widely used in the food industry as a thickener because of their compatibility with acidic emulsions and low cost on a viscosity basis. They provide a high degree of viscosity at low concentrations through entangled networks which restricts the movement of individual chains so trapping water, modifying texture and stabilising product consistency to changes in temperature (Fox, 1992). Galactomannans are increasing in popularity as both thickeners and functional food additives. A better understanding of the factors affecting the depolymerisation of galactomannans will allow more effective use in a wide range of heat treated food applications.

Locust bean gum (LBG) is derived from the seeds of the Carob tree (*Ceratonia siliqua*) which is indigenous to the Middle East. The gum is obtained by first removing the testa (husk) by roasting and then grinding the endosperm (Fox, 1992). Guar gum (GG), also known as guaran, is obtained from the seeds of the shrub *Cyamopsis tetragonobulus*, which is indigenous to North West India and Pakistan. The plant is an annual legume and seeds can be produced by conventional farming practices making it more economical than other seed gum sources (Whistler, 1993).

1.1. Properties of galactomannan

Galactomannans, water-soluble polysaccharides found in the seed endosperm of a variety of legumes, consist of a 1-4 linked β-D-mannopyranosyl (MAN) backbone partially substituted with (1-6)-α-D-galactopyranosyl (GAL) side groups (Dea & Morrison, 1975) (Fig. 1). One of the main differences between galactomannans from different sources is the degree and nature of galactose substitution. Guar gum contains approximately twice as many α-D-gal branches compared to locust bean gum. Values of mannose to galactose ratio (M:G) for gums from different sources can vary but values quoted are usually in the range of 3.2–4.0 for LBG and 1.4–2.0 for GG (Daas, Schols, & de Jongh, 2000; Srivastava & Kapoor, 2005). The galactose side chains tend to inhibit molecular associations and hence these variations in galactose content lead to differences in the functional properties of galactomannans, for example, solubility, thickening and gelation. A major difference is that GG can be dissolved in cold water, whereas LBG requires hot water for dissolution (Hui & Neukom, 1964). Guar gums have a regular blockwise distribution of galactose while LBG is found to have both random and ordered distribution (Daas et al., 2000). It is thought that association between the unsubstituted smooth mannan sequences form "hyperentanglements", causing GG and LBG to depart from the normal concentration dependent viscosity giving them higher viscosity at lower concentrations than many other galactomannans (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). However an additional possibility is that association also occurs between the galactose branches lying above and below each other with no nec-

* Tel.: +90 374 2541000x4219/538 5671006; fax: +90 374 2534558.
E-mail address: kok_s@ibu.edu.tr.

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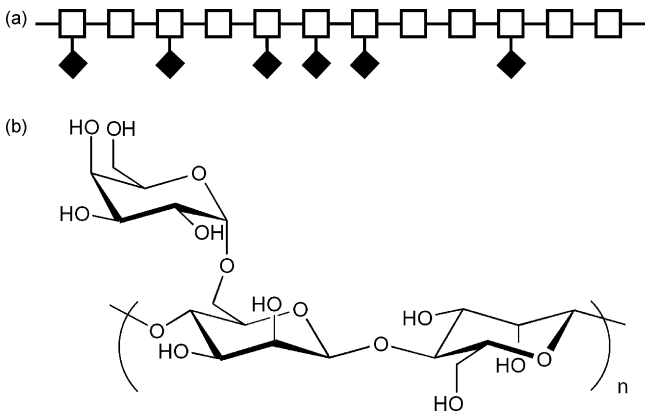

Fig. 1. Representation of galactomannans (a) schematic (Theander, 1986). (□) D-Mannose, (♦) D-galactose and (b) chemical structure.

essary involvement of the unsubstituted mannan backbone (Doyle, Lyons, & Morris, 2009).

1.2. Influences on viscosity

The viscosity of galactomannans in aqueous solutions depends on time, concentration, pH, ionic strength and degree of mechanical dispersion in preparation (Doublier & Launay, 1976; Kök, Hill, & Mitchell, 1996; Morris et al., 1981). The degree of solubility of the polysaccharide and the extent of degradation due to endogenous enzymes, bacterial contamination, acid hydrolysis, β-elimination or oxidative reductive depolymerisation (ORD), a reaction mediated by oxygen, a transition metal and a free radical, will also affect viscosity.

Seed gums are widely used in many food products including those that are heat treated, however the functionality of galactomannans can be reduced when heated. This is due to depolymerisation and hence a reduction in the average molecular weight of the polymers. When degradation was monitored by changes in viscosity on heating, LBG was shown to be less affected than GG. The activation energy for degradation of LBG was found to higher than that of GG and this is thought to be due to the greater ability of LBG to associate in solution (Kök, Hill, & Mitchell, 1999a). It is recognised that association will protect a polymer against degradation (Stokke, Christensen, & Smidsrod, 1992). The use of antioxidants in the food industry is common for the prevention of lipid oxidation, but they are not used to ensure reduction or elimination of thermal degradation of polysaccharides. GG and LBG have both been shown to contain significant levels of protein even when refined (Kök, 2007; Kök, Hill, & Mitchell, 1999b; Lazaridou, Biliaderis, & Izydorczyk, 2000). The protein component may play an important role since amino acids are known to be effective in scavenging free radicals which are involved in degradation (Bradley & Mitchell, 1988).

Previous research in this area has worked with mixed galactomannan systems (Mitchell, Hill, Kumel, Harding, & Aidoo, 1992). The objective of this research was to investigate the influence of pH and antioxidants, on the depolymerisation of pure GG and LBG galactomannans, at elevated temperatures. The research was conducted using a Bohlin Rheometer with a coaxial cylinder (cup and bob) C25 geometry and oscillation technique. This technique was chosen as it employs small alternating movements, especially at low shears, therefore does not disturb the network structure of the material being investigated (Sun & Gunasekaran, 2009; Weitz, Wyss, & Larsen, 2007).

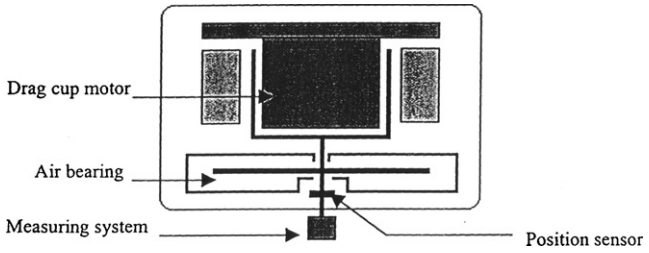

Fig. 2. Schematic diagram of Bohlin CS Rheometer.

2. Materials and methods

2.1. Sample preparation

Guar gum (G-4129 Lot 80HO34) and Locust bean gum (G-0753 Lot 82HO724) both analytical grade were obtained from Sigma Inc., UK. Sodium phosphate buffer was prepared from the stock solutions of Na_2HPO_4 and NaH_2PO_4 at 0.5 M concentration. The stock solutions were diluted to 100 mM concentration and pH was adjusted to 7.0 ± 0.1 by adjusting the mixture appropriately.

For the pH experiments values were adjusted between 5.0 and 9.0 in the same way. pH was measured using a WPA Linton CD620 (Cambridge, UK) digital pH meter at 25 °C. Solubilisation conditions were defined as an hour at 70 °C for LBG samples and 30 min at 40 °C for GG samples. This was achieved using a water bath with samples continuously stirred by a bench mixer (Griffin and George Ltd., UK) at low speed. These conditions were chosen, as they do not cause significant degradation (Kök et al., 1999a) Heat treatments up to 100 °C were carried out in media bottles (20 ml or 250 ml) using the same equipment as that used for solubilisation. A small autoclave (Benchtop-50, Harvard/LTE Ltd., England) was used to achieve high temperature treatment up to 121 °C. Retorted samples were contained in cans (250 ml or 500 ml). All treatments were carried out in triplicate.

2.2. Effect of pH

GG and LBG (300 ml) samples were prepared at 1% (w/v) in 100 mM sodium phosphate buffer at pH 5.0, 6.0, 7.0, 8.0 and 9.0 and left over night. The pH was established using the buffers of 100 mM $Na2HPO_4$ (pH: 9.15) and $NaH2PO_4$ (pH: 4.7). Duplicate samples were heated for 30 min at 100 °C and left over night.

2.3. Effect of antioxidants

GG and LBG samples (1%, w/v) were prepared in 100 mM sodium phosphate buffer at pH 7 at ambient temperature (20 °C) using a high shear Silverson mixer for 2 min. The antioxidants propyl gallate and sodium sulphite was added to both gums at a low level of 20 and 30 ppm, respectively, and a high level of 500 and 1000 ppm. Solutions were left to stabilise overnight before conducting viscosity measurements and heat treatment experiments. Controls without antioxidant were prepared in a similar manner. Samples were heated in a water bath for 30 min at 80 and 100 °C and an additional sample was autoclaved at 121 °C for 5 min. Samples with and without antioxidant were held at ambient temperature.

2.4. Standard measurement of viscosity

All measurements of viscosity were made with a Bohlin CS 10 Rheometer (Fig. 2).

Using oscillation technique as this causes less disruption to the polymers. In oscillation experimentation, the sample is subjected to a harmonic shear stress with controlled amplitude of "σ_0" and

angular frequency ω described by:

$$\sigma = \sigma_0 \cos \omega \times t \quad (1)$$

The resulting harmonic strain response γ can be written as:

$$\gamma = \gamma_0 cos(\omega \times t - \delta) \quad (2)$$

where γ_0 is the strain response, δ is a phase lag which is a characteristic of viscoelastic behaviour and t is time. Complex viscosity η^* can be related to the complex modulus (G^*) and the frequency (f) by:

$$\eta^* = \frac{G^*}{\omega} \quad (3)$$

where

$$\omega = 2\pi \times \mathrm{f} \quad (4)$$

A coaxial cylinder (cup and bob) C25 geometry was chosen because it is suitable for working with low viscosity materials and fluid suspensions. The diameter of the cup is in proportion to the bob size as defined by the DIN Standard. The large surface area of the C25 gives it a greater sensitivity and consequently it will produce good data at low shear rates and viscosities. In addition, it is tolerable to material containing particulates, not critical on eccentricity and misalignment, has good temperature control and gives a high level of data repeatability. Therefore it was concluded that for oscillatory measurements at high frequencies on low viscosity materials, the C25 cup and bob geometry with a small gap would produce the optimum test conditions.

2.4.1. Linear viscoelastic region (LVER)

The theory used to calculate viscoelastic parameters from oscillatory measurements employed in the software by modern rheometers assumes linear behaviour, that is to say that stress is proportional to strain under all conditions. Provided that the applied stress is kept small enough, the strains produced will indeed follow this behaviour.

Thus any dynamic tests on an unknown sample must start with a stress amplitude sweep. This is achieved by oscillating at a fixed frequency and slowly increasing the applied stress. The measured values for the moduli and viscosity will remain constant. When the applied stress becomes too great, the induced strain will start to cause the material "rupture", i.e. some flow will be obtained on top of the deformation. This can be observed as the measured value of elasticity falling, whilst the measured viscous component starts to increase. In order to stay within the LVER, working conditions must be at stresses below this point. Having determined the amplitude that keeps tests of a particular sample safely within the LVER, further tests can then be carried out using the frequency sweep to measure the sample's viscoelastic behaviour.

Before all experiments the LVER was established using Stress Sweep techniques at different frequencies. The LVER was used to define a target strain for the oscillation experiments. The values reported for complex viscosity represent the mean of three replications obtained for at least two repetitions of each treatment for pH and antioxidant effect.

Viscosity was measured for both gums using the Oscillation method, frequency of 0.01–10 Hz and the target strain set at 0.5, which is a dimensionless quantity, using C25 geometry measuring device. Results are given as the mean of triplicate measurements of duplicate treatments. Standard deviation values are given for each figure. Results are reported at 0.1 Hz because at this frequency there will be the least shear disturbance on the material.

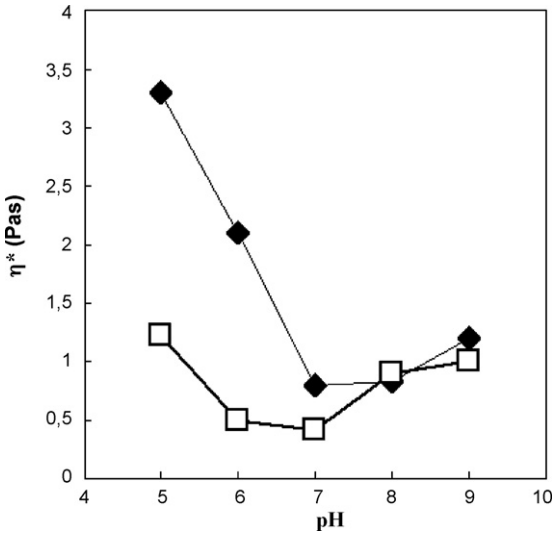

Fig. 3. Complex viscosity of GG (♦) and LBG (□) at pH ranging from 5 to 9, heated at 100 °C for 30 min. Measurements made at 25 °C, frequency of 0.1 Hz, target strain of 0.5 (SD < ±2.5%).

3. Results and discussion

3.1. Effect of pH on thermal degradation

Galactomannans are neutral and should not be expected to change viscosity in a range of buffers except at very high pH, where it has been reported that hyperentanglements are suppressed and neutralisation gives a slow recovery of viscosity for GG and LBG (Goycoolea, Richardson, Morris, & Gidley, 1995). It is the ionisation of hydroxyl groups which causes a dissociation of the hyperentanglements, by electrostatic repulsion, and subsequent decrease in viscosity. In experiments with fenugreek galactomannan, which has a higher galactose content than GG or LBG, this effect was seen to be rapidly reversed on neutralisation with a salt (Doyle et al., 2009).

In the narrower range of pH 5.0–9.0 investigated in this work there was no significant change in viscosity of gum solutions at ambient temperature. However subjecting the same solutions to heat treatment at 100 °C for 30 min did show differences in viscosity indicating that degree of thermal degradation is influenced by the solution pH (Fig. 3). Michel, Doublier, and Thibault (1982) also found an interaction between temperature and pH in the rate of depolymerisation of galactomannans. However, in contrast to the findings of this research, they found loss of viscosity to be greater at pH below 4.5. Bradley (1989) has also shown that 0.5% GG is more shear thinning in acid than in neutral conditions at temperatures above 90 °C.

The results of this research show a very different response, with the greatest loss in viscosity being exhibited at pH 7.0 by both GG and LBG (88.3% and 76.3%, respectively; Table 1), and the least loss of viscosity at pH 5.0 (48.4% and 34.2%). These results therefore suggest that there is greater thermal degradation at neutral pH than acidic which could be attributed to free radical attack being optimum at this pH. Solutions of GG and LBG made with deionised water, as opposed to buffer, without any adjustments for pH show low pH values of 2.76 and 2.48, respectively (Yaseen, Herald, Aramouni, & Alavi, 2005). The ability of GG to maintain viscosity at low pH and high temperatures, in food products, has been demonstrated. It was found to effectively stabilise tomato ketchup, with a pH value of 3.0, which exhibited almost no serum loss after 120 days storage at 50 °C (Gujral, Sharma, & Singh, 2002). Therefore, if these gum solutions are naturally acidic, adjusting the pH

Table 1
Decrease in complex viscosity (η^*) of 1% GG and 1% LBG solutions at pH range 5.0–9.0, heated at 100 °C for 30 min. Viscosity of non-heat treated samples: GG = 6.4 Pa s and LBG = 1.9 Pa s.

	Decrease in η^* (Pa s)		%Decrease in η^*	
pH	LBG	GG	LBG	GG
5.0	0.65	3.10	34.2	48.4
6.0	1.40	4.30	73.7	67.2
7.0	1.45	5.65	76.3	88.3
8.0	1.00	5.50	52.6	85.9
9.0	0.90	5.15	47.4	80.5

Measurements made at 25 °C, frequency of 0.1 Hz, target strain of 0.5; using C25 geometry. Results are given as mean of duplicate measurements.

towards neutral may influence their stability. There is no evidence of this at ambient temperature but heat treatment reveals a greater susceptibility to degradation.

Alternatively the low viscosity readings achieved at pH 7 could be due to the degradation effect of a naturally occurring enzyme. However, depolymerisation caused by enzymes seems less probable in this research due to the high temperature involved and because storage at ambient does not seem to show degradation.

3.2. The effect of antioxidants on thermal degradation

The antioxidants propyl gallate and sodium sulphite are both permitted food additives and have been used in previous experimental work with galactomannans. It has been shown that the use of propyl gallate and sulphite, at a ratio of approximately 1:3, reduces the loss in viscosity which occurs on heating galactomannans in mixed gels (Mitchell et al., 1992). Ferulic acid, as an alternative to propyl gallate was used with sodium sulphite at an optimal ratio of 1:1.7 and found to maintain viscosity of guar, heated to 121 °C for 60 min, at 70% of pre-treatment values (Hill & Gray, 1999). Addition of sulphite at levels between 0.004% and 1% was found to protect guar from thermal degradation with higher levels producing a more viscous guar post-retorting (Paterson, Hill, Mitchell, & Blanshard, 1997).

In this research the antioxidants propyl gallate and sulphite were added at a concentration of 0.05% and 0.1% (ratio of 1: 2) to both GG and LBG. Typically the sulphite is thought to act as an oxygen scavenger, removing dissolved oxygen and so reducing the production of free radicals. Propyl gallate however acts as a free radical terminator and there is strong synergistic interaction between the two which seems particularly evident with galactomannans (Mitchell, Reed, Hill, & Rogers, 1991).

As expected, at ambient temperature viscosity change is not observed, even on addition of high levels of antioxidants. When subjected to heat treatment, the gum solutions with a low level of antioxidant addition showed similar decreases in viscosity to solutions without antioxidants.

However, when the addition of antioxidants was increased to high levels, the effect on viscosity, observed at temperatures of 100 and 121 °C, was significant (Fig. 4a and b). As in previous work with these gums (Kök et al., 1999a) the response of LBG to heat treatments differed from that of GG. The initial rise in viscosity of LBG between 20 and 80 °C is because it is reaching its fully solubilised state, yet viscosity at this temperature is slightly improved with antioxidant addition suggesting that some thermal degradation is taking place even at 80 °C and may be occurring because of the length of time (1 h) the solutions were held. Over the same temperature range GG shows 44% decrease in viscosity. On heating to 100 °C, GG shows 95% loss in viscosity, in contrast to the findings of Hill and Gray (1999) who only found 30% loss in viscosity. Interestingly, LBG has only 15% loss in viscosity, when heated to 100 °C with antioxidants, compared with 74% loss when no antioxidants

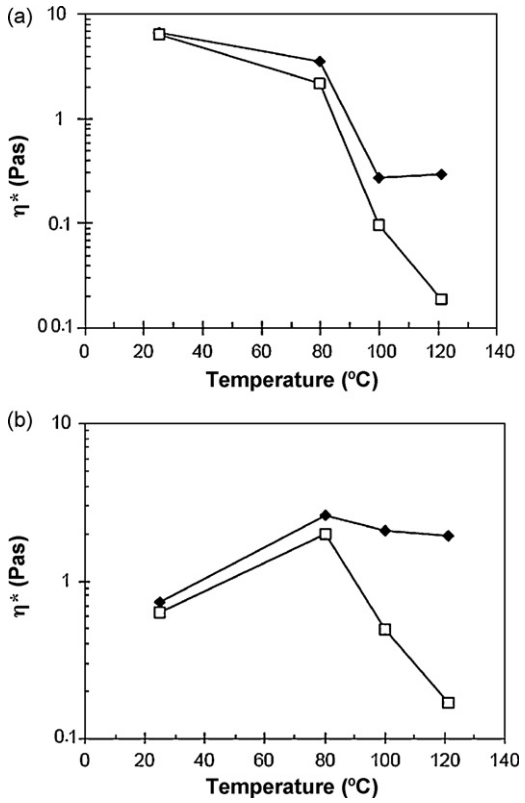

Fig. 4. Complex viscosity of (a) 1% GG and (b) 1% LBG heat treated for 30 min at temperatures ranging from 25 to 121 °C with (♦) and without (□) antioxidants, sodium sulphite (1000 ppm) and propyl gallate (500 ppm). Measurements made at 25 °C, frequency of 0.1 Hz, target strain of 0.5 (SD values are <±3.5%).

are added. The protective effect is continued up to 121 °C where drop in viscosity is 33% and 91%, respectively, in solutions of LBG with and without antioxidants. It is already recognised that LBG shows a greater resilience to thermal degradation, than GG, due to its greater intermolecular associations (Kök et al., 1999a). These findings show that the prevention of thermal depolymerisation by antioxidant addition is also much more significant for LBG than GG. This could be due to the antioxidants encouraging the intermolecular associations, by preventing the ionisation of hydroxyl groups, which would otherwise cause a dissociation of the hyperentanglements (Doyle et al., 2009).

4. Conclusion

Effectiveness of galactomannans as food thickeners and stabilisers is dependent on their resilience to depolymerisation especially thermal. They are susceptible to degradation by oxidative reductive depolymerisation reactions, which causes viscosity to be lost. The rate of loss is dependent on temperature and pH. This research is a contribution to understanding the effect of pH and antioxidants on the rate of pure analytical grade GG and LBG depolymerisation.

In this research it was confirmed that varying pH at ambient temperatures did not influence viscosity of galactomannan solutions. However a combination of varying pH and heat treatment up to 100 °C caused significant loss of viscosity. Surprisingly the maximum loss of viscosity, 88.3% for GG and 76.3% for LBG (Table 1), were obtained at pH 7.0. This may be indicative of free radical attack being optimum at this pH or perhaps induction of naturally occurring enzymes. The antioxidants provide LBG with significantly greater stability at higher temperatures. This suggests that it is the action of free radicals at higher temperatures that causes depolymerisation and the antioxidants are working to preserve

intermolecular associations in LBG. However other forces must come into play as, even at saturated levels of antioxidant addition, the degradation of gums can be prevented only to an extent. The mechanisms underlying the relationship between pH, temperature and viscosity need to be further investigated at molecular level in order to establish whether disassociation of hyperentanglements or depolymerisation is more significant at high temperatures.

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