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Modulation in the rheological behaviour of porcine pepsin treated guar galactomannan on admixture with κ-carrageenan

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ABSTRACT

The non-specific action of pepsin on guar galactomannan (GG) resulted in selective removal of galactose residues, leading to the formation of galactose – depleted guar galactomannan (GDGG) with decrease in Mw and change in G: M ratio of 1:3.4, thus mimicking that of locust bean gum (LBG). Admixture of GDGG with κ-carrageenan revealed a two fold increase in the magnitude of elasticity (G') compared to κ-carrageenan alone, suggesting a synergistic interaction similar to LBG with κ-carrageenan. Blends of GDGG and LBG with κ-carrageenan at a concentration of 40% (w/w, 0.8% total biopolymer) showed a maximum increase in G'. The GDGG/κ-carrageenan blend also showed a T_m of 47 °C, similar to LBG/κ-carrageenan blend. Thus, debranching of guar galactomannan by the catalytic action of pepsin is beneficial for improved functional properties and diversified applications.

1. Introduction

Guar galactomannan (GG) derived from the seeds of endospermic leguminous plant *Cyamopsis tetragonaloba* L., is a highly viscous heteropolysaccharide having β-1,4-D-mannopyranose backbone and alternative single side chain of α-1,6-D-galactopyranose residues with G:M ratio of 1:2. GG finds wide spread applications based on its ability to modulate rheological properties, thicken and stabilize many food products, as functional food ingredients and also as a source of dietary fiber (Greenberg & Salvin, 2003). In pharmaceutical sector its functional properties are of importance for controlling the release of drugs to gastrointestinal tract (Chourasia & Jain, 2004) and for transdermal drug delivery system (Badmapriya & Rajalakshmi, 2011). Also GG is the material of choice in various other industries like textile (Kokol, 2002), oil recovery (Ebinger & Hunt, 1989), mining (Rath, Subramanian & Laskowski, 1997), cosmetics (Bergfeld, 2012), etc. The blend of galactomannan with other (biocompatible) polysaccharides such as xanthan and carrageenan brings about synergistic interaction by binding with their helices forming gelation of the two components resulting in the improvement of product quality and reduction in production cost, which are the main contributing factors for their industrial applications. Specifically, the blends of galactomannans such as guar and locust bean gum (LBG – composed of β-1,4-D-mannopyranose backbone and single side chain of α-1,6-D-galactopyranose residues with G:M ratio of 1:4) are used in combination with xanthan and carrageenan in a range of applications that include coatings, drug delivery, oil/gas production and food additives (Fox, 1992). Admixture of LBG with carrageenan induces a strong synergistic gelling interaction, whose rheological behaviour is well established (Martins, 2012). On the contrary, the addition of GG to carrageenans only increases the viscosity of the solution without gelation (Tako & Nakamura, 1985). The latter is mainly attributed to the greater proportion of side chain galactose residues on the mannan backbone, which do not to bind with the helices of xanthan or carrageenan.

Inspite of various functionalities, high viscosity of GG due to a higher degree of substitution by galactose residues restricts its wide spread application potential. Therefore, modification of GG by a selective removal of galactose residues is advantageous for its diversified applications in various food and non-food industries. The structural modification of GG using α-galactosidase enzyme has been widely studied for its extended range of industrial applications (Ganter, Sabbi, & Reed, 2001). The α-galactosidase treated GG forms a hydrogel on complexation with helical polysaccharides like xanthan, which is of considerable interest in food and pharmaceutical applications (Chidwick, Dey, Hart, McKenzie, & Pridham, 1991). Yamatoya (1994) showed the enzyme-modified guar gum

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with low molecular weight exhibiting beneficial effect as water - soluble dietary fiber.

Investigations by various groups though demonstrated that the fine structure of GG can be altered by employing highly specific enzymes for the removal of galactose residues which will subsequently enhance its synergistic interaction with other biocompatible polysaccharides (Chidwick et al., 1991; Kloek, Luyten & van Vliet, 1996), the high cost involved for the isolation of these enzymes and their laborious production steps are non-economical, and have paved the way to look for alternative approach such as use of unrelated enzymes which are able to selectively debranch (non-specifically) the GG without depolymerising the core mannan backbone. Nevertheless, the recent literature reveals the non-specificity of various enzymes belonging to hydrolases, such as cellulase, lipase, hemicellulase, lysozyme, pectinase (Xia, Liu & Liu, 2008; Lee, Xia & Zhang, 2008) and a few proteases like pepsin, papain, pronase, bromelain on chitosan (Muzzarelli, Terbojevich, Muzzarelli, & Francescangeli, 2002; Kittur, Vishu Kumar & Tharanathan, 2003; Kumar, Varadaraj, Gowda & Tharanathan 2007) and other polysaccharides. Our recent findings on pepsin – a proteolytic enzyme catalyzing the hydrolysis of GG (Shobha, Gowda & Tharanathan, 2014) showed an alternative way to produce GDGG with reduction in galactose content. Hence, the main objective of the present study involves the use of pepsin in debranching GG to obtain modified GG with an altered G: M ratio, which may possibly mimic the rheological properties of LBG on co-gelation with κ-carrageenan. Possibly such an approach may assume greater significance in view of product development requirements of the food and other allied industries.

2. Materials and methods

Guar gum was procured from Hindustan Gum Chemicals, Haryana, India, Pepsin (porcine stomach mucosa, E.C. 3.4.23.1), locust bean gum, κ-carrageenan (with K^+ ions) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other reagents and chemicals were of highest purity.

2.1. Preparation of galactomannan solution

GG and GDGG solution (1%, w/v) was prepared by slow addition of the gum powder to deionised water at room temperature with continuous stirring for 12 h at 80 rpm to enhance complete hydration of the gum and to attain maximum viscosity.

LBG solution (1%, w/v) was prepared by adding the gum into hot water and further heating to 80 °C for about 30 min with continuous stirring to ensure complete solubility and to obtain a clear homogenous solution. Sodium azide (0.1% solution) was added to prevent bacterial contamination.

2.2. Preparation of κ-carrageenan solution

κ-Carrageenan (1%, w/v) was prepared by dispersion of gum in deionised water with stirring on hot water bath at 80 °C for 30 min followed by cooling at room temperature for about 2 h. Before cooling the solution was centrifuged at 5000 rpm for 15 min to remove the entrapped air bubbles along with the undissolved particles.

2.3. Preparation of GDGG

GG solution (0.5%) together with purified pepsin in the enzyme: substrate ratio of 1:100 (w/w) was incubated for different periods (1–10 h) at optimum conditions of pH 5.5 and 40 °C (Shobha, Gowda & Tharanathan, 2014) followed by termination of the reaction by heating and adding three volumes of ethanol (95% v/v). After centrifugation (10,000 rpm, 15 min) the GDGG sediment was washed thoroughly with alcohol and freeze dried (Shobha & Tharanathan, 2008).

2.4. Characterization of GDGG

The molecular mass (Mw) of GDGG was studied by two techniques, namely (a) viscometric and (b) GPC methods

2.4.1. Viscometry

Viscosity of GDGG dissolved in water (0.1–0.5%) was measured using Ostwald viscometer at 25 °C. The Mw was calculated using Mark–Houwink's equation,

$$\eta = K \times (\text{molecular mass})^{\alpha} \quad (1)$$

where η = intrinsic viscosity, $K = 3.04$ and $\alpha = 0.747$ (Cheng, Brown & Prudhome, 2002).

2.4.2. Gel permeation chromatography (GPC)

GDGG solution (1 mL, 0.5%) was loaded onto Sepharose CL-4B (Bed volume, 120 mL) and eluted with water at a flow rate of 18 mL h^{-1}. Each fraction was analyzed for total sugar by phenol-sulphuric method (Rao & Pattabiraman, 1989). The column was calibrated with T-series dextran standards.

2.4.3. HPSEC-RI

HPSEC was performed on Shimadzu LC 8A system connected to RI detector, using E-linear and E-1000 columns (Waters Associates, Millford, USA) connected in series with distilled water as the mobile phase at a flow rate of 0.6 mL min^{-1}.

2.4.4. Gas liquid chromatography (GLC)

The galactomannan content and galactose/mannose (G/M) ratio of GG, LBG and GDGG was evaluated, after acid hydrolysis followed by derivatization into alditol acetate, by GLC on OV-225 (3% on Chromosorb W) column connected to Shimadzu 8A equipped with flame ionization detector (Sawardekar, Sloneker & Jeanes, 1965) at 200 °C with nitrogen as carrier gas.

2.5. Preparation of κ-carrageenan/galactomannan blends

κ-Carrageenan/galactomannan mixed gels (0.8%, total biopolymer) were prepared in a ratio of 1:1 (w/w) by dispersing in deionised water under constant stirring for about 30 min. Then the mixture was heated to 60 °C for 15 min and centrifuged at 5000 rpm for 15 min to remove the entrapped air bubbles. The solution was poured into a gel jar and allowed to set at room temperature for 24 h.

2.6. Rheological measurements

Rheological measurements were performed on RV Rheometer-SR-5 (Perkin Elmer) fitted with a probe of 25 mm diameter using parallel plate geometry for both individual and blends of polymers. Elastic or storage (G') and viscous or loss moduli (G'') were measured in the linear domain by dynamic frequency test with the frequency range of 0.1–100 rads/s. Dynamic stress sweep test at 1 Hz were performed in the range of 10–1000 Pa to determine critical shear stress for the onset of non-linear viscoelastic behavior. This critical shear stress has been interpreted as the yield stress of the blends (Pai & Khan, 2002). All the experiments were carried out at 25 °C.

The effect of κ-Carrageenan and effect of mixing temperature on the co-gelation with GDGG was studies by varying the concentration from 0% to 80% (w/w, 0.8% total biopolymer) and 10–50 °C.

Table 1
Characteristics of GDGG.

	Incubation time (h) with pepsin	Mannose (%)	Galactose (%)	G:M ratio	Molecular weight (kDa)		
					Viscometry	GPC	HPSEC
Native GG	0	68	33	1:2.0	240	239	251
Locust bean gum	0	80	20	1:4.0	170	168	179
GDGG	3	77	23	1:3.4	184	180	190

2.7. Texture profile analysis

Textural parameters, viz. hardness and cohesiveness of mixed gels (45 mm diameter × 35 mm height) were measured by Instron Texture analyzer equipped with a load cell of 50 N, 35 mm probe, speed 20 mm min^{-1} followed by double compression (50%). The samples were cooled to 20 °C before taking measurement.

2.8. Differential scanning calorimetry

Differential scanning calorimetric studies were performed on Micro DSC III (Waters) supported by Dac software. The temperature in the DSC cell was controlled at a programmed rate using proportional integral derivative control. The DSC cell was purged with liquid nitrogen at a rate of 40 mL min^{-1} and each run was carried out at a scan rate of 5 °C min^{-1} from 10 to 100 °C. About 15–20 mg of sample (κ-carrageenan (0.8%, w/v) and galactomannan/κ-carrageenan blends (w/w, 0.8%, total biopolymer, ratio 1:1) were weighed into an aluminium crucible and hermetically sealed with an aluminium lid using an encapsulating press.

2.9. Statistical analysis

The statistical analysis was performed on Graph Prism 6.0 with Bonferroni multiple comparison test with significance $P < 0.05$. The samples were run in triplicates.

3. Results and discussion

3.1. Characterization of GDGG

The decrease in Mw of GDGG as determined by viscometry and GPC was in good agreement with each other (Table 1). A steady decrease in Mw (due to the removal of galactose residues) reflected by the change in the G: M ratio (determined by GLC) of GDGG (Table 1) upon incubation from 1 to 10 h also indicated the non-specific catalysis (debranching) of GG by pepsin. Appearance of a single peak in GPC and HPSEC indicated the homogeneity of GDGG (data not shown). The latter obtained after 3 h of incubation with pepsin showed a galactose:mannose ratio of 1:3.4, thus mimicking that of LBG.

3.2. Rheology of galactomannan solutions

Native GG solution (Fig. 1A) at 1% concentration exhibited a typical viscous behaviour with viscous modulus G'' dominating G' in the low frequency range followed by the domination of the latter with a cross point ($G' = G'$ at 12–15 Pa) at high frequency range (10 rad/s). GDGG solution (1%, w/v; Fig. 1B) with depleted galactose residues also showed a similar property, which means that the removal of galactose residues did not alter the gross rheological behaviour of galactomannan. The unaltered gross rheological behaviour is consistent with the fact that enzyme strips off only the galactose residues without effecting the hydrodynamic radius of the guar galactomannan (Pai & Khan, 2002).

The rheology of LBG (1%, w/v; data not shown) solution showed G'' dominating G' but without a cross point which is frequency dependent, thus suggesting the behaviour of a dilute polymer solution. In comparison, κ-carrageenan (1%, w/v) solution (Fig. 1C) exhibited a higher G' over the entire frequency range, indicating the behaviour of a firm gel with strong network.

3.3. Galactomannan/κ-carrageenan blends

Fig. 2A depicts the viscoelastic behaviour of LBG/κ-carrageenan blend (0.8%, w/w, total biopolymer concentration, 1:1 ratio). It showed a significant increase in the elastic (G') and viscous moduli (G'') independent of frequency as compared to carrageenan alone (G' & $G'' = 10^4$ & 10^3 Pa). A two-fold increase in elasticity ($G' > G''$) over the entire frequency range corresponded to the characteristic feature of a true gel, indicating a synergistic interaction. Clark and Ross-Murphy (1987) also reports a similar synergistic interaction with increased elasticity leading to true gels formation. Cairns, Miles, Morris, and Brownsey (1987) interpret such an interaction as due to the entrapment of LBG within the κ-carrageenan network.

3.4. Interaction of GDGG with κ-carrageenan

Fig. 2C show the viscoelastic behaviour of GDGG/κ-carrageenan (0.8%, w/w total biopolymer, 1:1 ratio) blends with $G' > G''$ over the entire frequency range, indicating the co-gelation of the biopolymers. Shobha and Tharanathan (2009) shows the decrease in the galactose content of GG associated with the increased synergistic behaviour upon co-gelation with xanthan. A two fold increase in the magnitude of G' in the plateau region with decrease in tan δ suggest a gain in elasticity, a characteristic feature for forming more strong and elastic gels (Schorsch, Garnier, & Doublier, 1997) in comparison to κ-carrageenan alone (G' of 10^4 Pa). Buchard and Ross-Murphy (1990) describe the appearance of plateau in the elastic moduli region over the entire frequency range with the magnitude of more than one order for true gels which are self-standing.

In comparison, GG/κ-carrageenan blend showed a different viscoelastic behavior (Fig. 2B), which resulted in $G' > G''$, with an increase in G'' over the entire frequency range. The magnitude of G' was similar to that of native κ-carrageenan solution (G' of 10^4 Pa), suggesting the role of the latter for increase in elasticity. Lopes, Andrade, Milas and Rinaudo (1992) report a similar increase of viscosity in blends of GG and xanthan due to molecular interaction. The enhanced synergistic interaction of GDGG is mainly attributed to the intermolecular interaction of galactose-depleted region of galactomannan backbone with the double helices of carrageenan forming secondary network (Fernandes, Goncalves & Doublier, 1991). Stading and Hermansson (1993) also reports the enhanced rheological behaviour of mixed gels of LBG/κ-carrageenan blends due to the synergistic molecular interaction of unsubstituted region of galactomannan with the double helices of carrageenan forming tight junction zones leading to secondary network.

Measurement of yield stress is one of the parameters for describing the textural qualities of gelling materials for commercial exploitation. Yield stress is defined as the critical stress at which the catastrophic breakdown of the structure skeleton occurs, leading to the observable flow of the material. Table 2 describes the

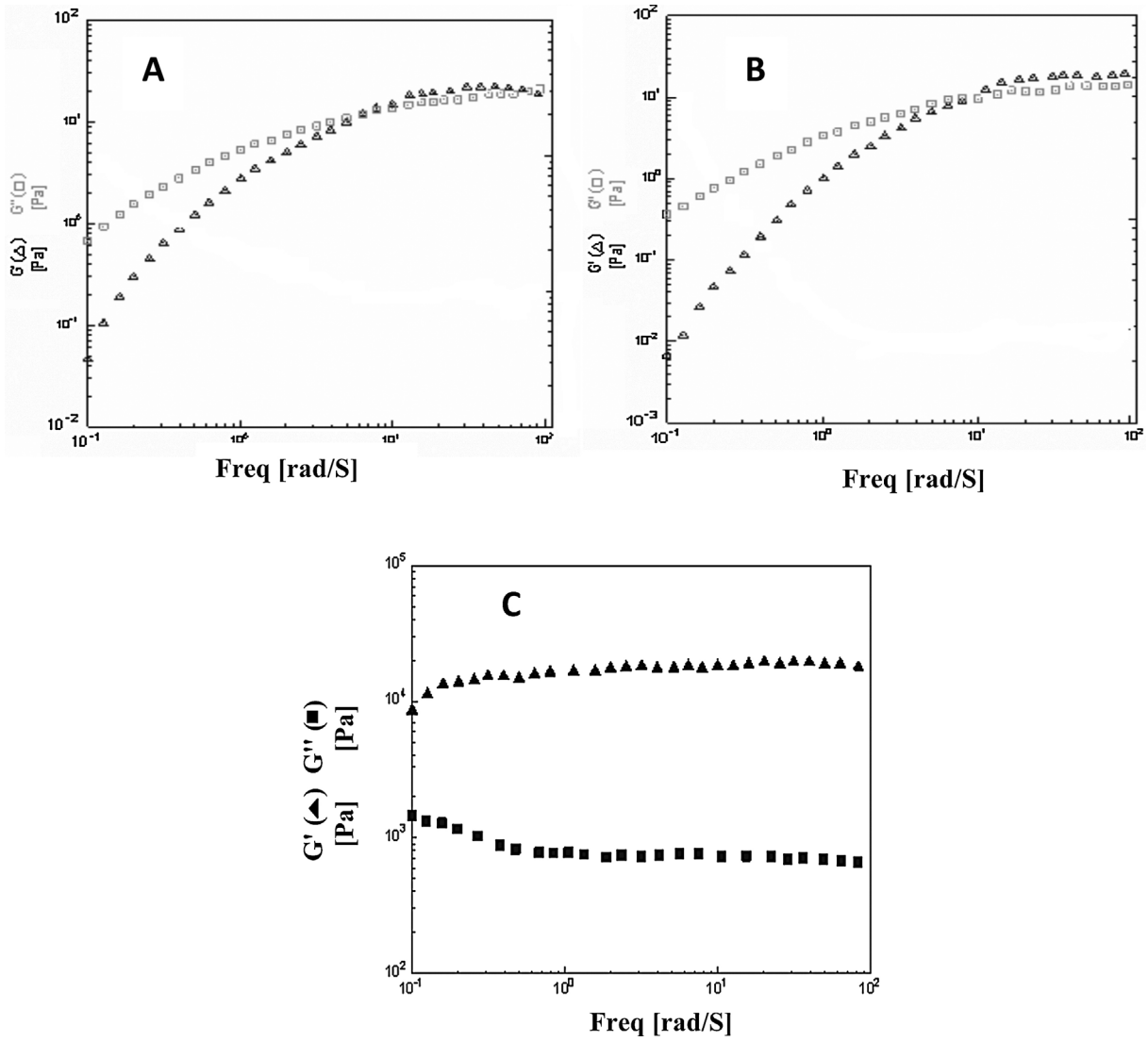

Fig. 1. Rheological behaviour of galactomannan solutions at 1%, w/v (A) Native GG, (B) GDGG & (C) κ-carrageenan solutions by dynamic frequency test method at frequency range of 0.1–100 rad/s.

yield stress exhibited by the native and modified GG blends as measured by the dynamic stress-sweep method. The GG blend with κ-carrageenan showed a very low yield stress compared to LBG blends. A sharp decrease in the G' region was observed at lower range (10–100 Pa), which may be due to rupture in the gel network, suggesting a weak association of GG. Whereas LBG blends showed highest yield stress of 1450 ± 17.24 Pa with the rupture point at a very high range showing its strong interaction.

The GDGG showed the capacity to withstand the stress of higher range, thus mimicking LBG blends. Luyten, Kloek, and van Vliet (1994) report a similar increase in the yield stress of the xanthan/enzyme modified galactomannan blend in comparison to xanthan/LBG blend. An increase in the strength of GDGG blend is attributed to the enzymatic removal of galactose residues from guar molecules with the exposure of unsubstituted mannan backbone to interact with κ-carrageenan forming junction zones, thus giving rise to strong gels.

Table 2
Yield stress of galactomannan/κ-carrageenan blends.

Blend	Yield stress (Pa)
κ-Carrageenan/native GG	450 ± 10.00
κ-Carrageenan/LBG*	1450 ± 17.24
κ-Carrageenan/GDGG*	1410 ± 11.14

* $P < 0.05$ vs κ-carrageenan/native GG.

3.5. Effect of κ-carrageenan concentration

Fig. 3A shows the effect of varying κ-carrageenan concentration on the rheological behaviour of galactomannan blends. Various concentrations of κ-carrageenan (0–80%, w/w) on both GDGG and LBG blends showed increase in the magnitude of G'. Though an increase in the elasticity was observed, κ-carrageenan at a concentration of 40% (w/w, 0.8% total biopolymer concentration) showed a maximum increase in the magnitude of elasticity (G'). A report from Fernandes, Goncalves and Doublier (1991) also suggests similar synergistic interaction as the concentration of κ-carrageenan increases, displaying maximum elasticity (G'). The κ-carrageenan/GDGG blend mixed at 40% κ-carrageenan concentration (w/w, 0.8% total biopolymer concentration) showed a maximum increase in magnitude with high yield stress (Fig. 3B).

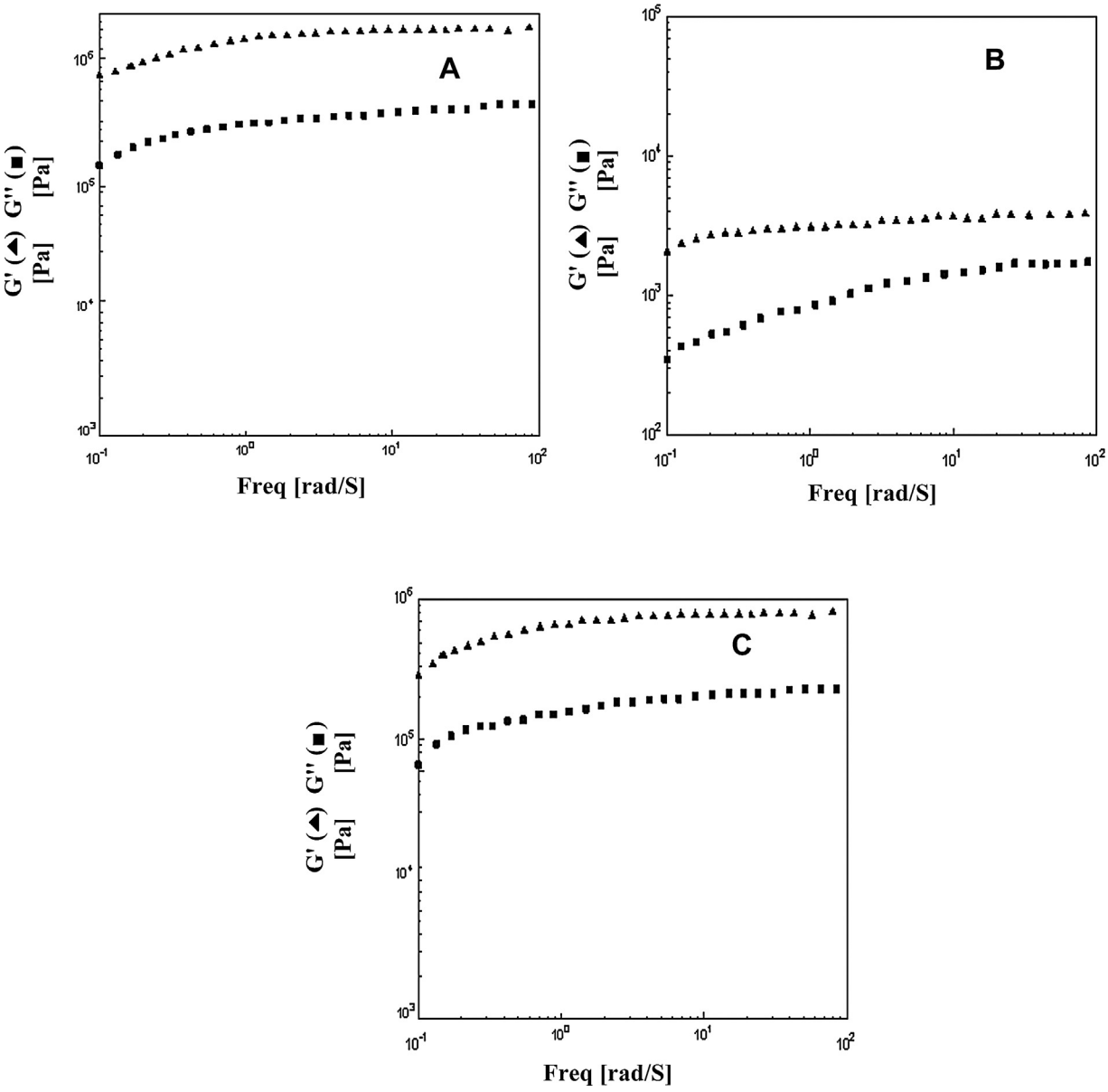

Fig. 2. Comparison of viscoelastic behaviour of blends at 0.8% (w/w, total biopolymer concentration at 1:1 ratio) (A) LBG/κ-carrageenan, (B) GG/κ-carrageenan (C) GDGG/κ-carrageenan by dynamic frequency test method at frequency range of 0.1–100 rad/s.

3.6. *Effect of temperature of mixing*

Fig. 4 shows the effect of variation of temperature on the synergistic interaction of galactomannan/κ-carrageenan blends. The κ-carrageenan/LBG blend showed an increased elastic modulus indicating the gelling characteristics. The mixture showed a synergistic interaction at 30 °C which was slightly higher than that of κ-carrageenan alone. GDGG on admixture with κ-carrageenan also displayed a similar increase in the elasticity as the temperature increased to 30 °C. The above results are consistent with that reported by Fernandes, Goncalves, and Doublier (1991a) suggesting a similar gelation mechanism.

The κ-carrageenan (0.8% w/v) showed a T_m of 33 °C (Table 3) in accordance with the previous report (Fernandes, Goncalves & Doublier, 1992). Upon heating, the hydrogen bonds are broken and double helices change their conformation giving rise to fusion of aggregates with the break up of network. The difference between melting temperature and gelling temperature is known as "thermal hysteresis" which is associated with the aggregation of double helices leading to gel formation (Mangione, Giacomazza, Bulone, Martorana, & San Biagio, 2003). The κ-carrageenan/LBG blend had a T_m of 48 °C. Similarly GDGG/κ-carrageenan blend also showed increase in T_m similar to LBG/κ-carrageenan blend. This increase is mainly attributed to the increase in the gel rigidity which is induced by the addition of galactomannan.

Table 3
T_m and ΔH of galactomannan/κ-carrageenan blends.

Blend	T_m (°C)	ΔH (J/g)
κ-Carrageenan	33	25.65
κ - Carrageenan/native GG	36	25.16
κ - Carrageenan/LBG	48	22.89
κ - Carrageenan/GDGG	47	22.95

Table 4
Texture profile analysis of polysaccharide blend.

	Hardness (N)	Cohesiveness	Springeness (mm)	Chewiness (N, mm)	Adhesiveness (N)	Gumminess (N)
LBG/κ-carrageenan	10.59 ± 1.09	0.069 ± 0.003	9.45 ± 0.821	6.67 ± 1.12	0.043 ± 0.05	3.4 ± 0.52
GG/κ-carrageenan	NG[a]	NG[a]	NG[a]	NG[a]	NG[a]	NG[a]
LBG	NG[a]	NG[a]	NG[a]	NG[a]	NG[a]	NG[a]
Carrageenan	2.8 ± 0.65	0.025 ± 0.004	4.26 ± 0.47	2.73 ± 0.77	0.012 ± 0.003	1.03 ± 0.075
GDGG/κ-carrageenan	8.9 ± 7.0	0.049 ± 0.07	8.85 ± 0.35	5.21 ± 0.50	0.057 ± 0.005	2.95 ± 0.510

[a] No gel formation.

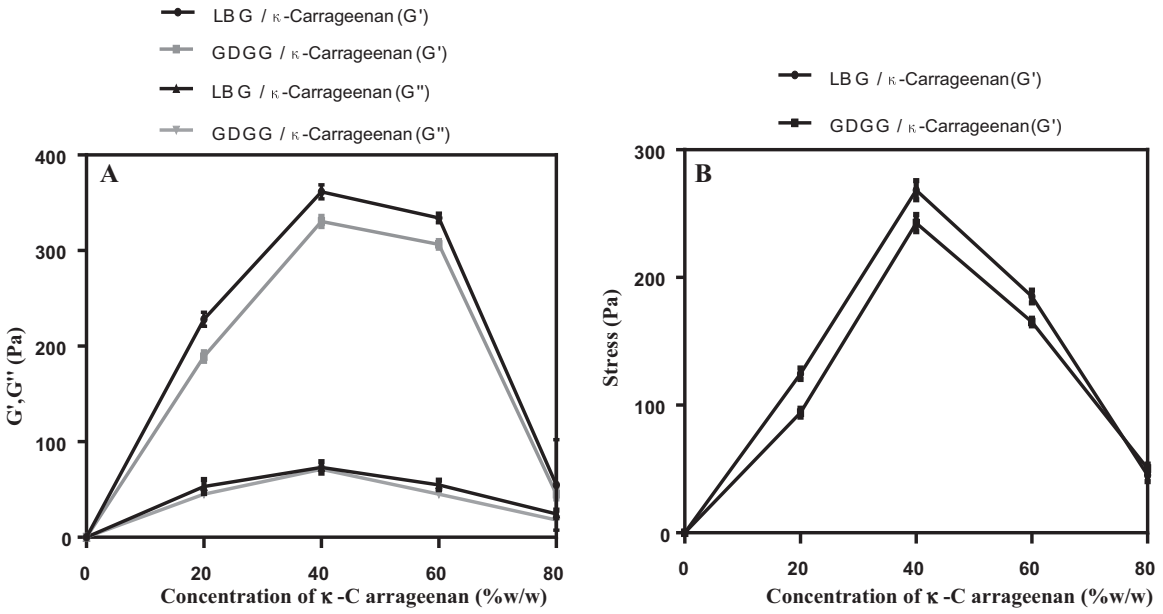

Fig. 3. Effect of κ-carrageenan concentration on gelation with galactomannans at 1 rad/s (A) dynamic frequency test, (B) dynamic stress – sweep test.

3.7. Texture profile analysis of polysaccharide blends

The synergistic behaviour exhibited by GDGG was also studied by texture profile analysis (Table 4) on co-gelling with κ-carrageenan. The native GG blends did not show any significant gelling, whereas the LBG blends showed synergistic interaction of the polysaccharides by strong gelation, thereby regulating the textural parameters namely hardness, cohesiveness, adhesiveness, gumminess, etc. GDGG with varying galactose content also showed a good gelling synergy with κ-carrageenan, when compared to native κ-carrageenan (0.8% w/v) alone, and having texture parameters akin to those of LBG blends with improved hardness, cohesiveness and gumminess in comparison to native GG. Brooks, Philip, Cooney, and Horgan (2000) also reports the enhanced synergistic behaviour of α-Galactosidase-treated guar galactomannan upon co-gelation with xanthan influencing the above textural parameters.

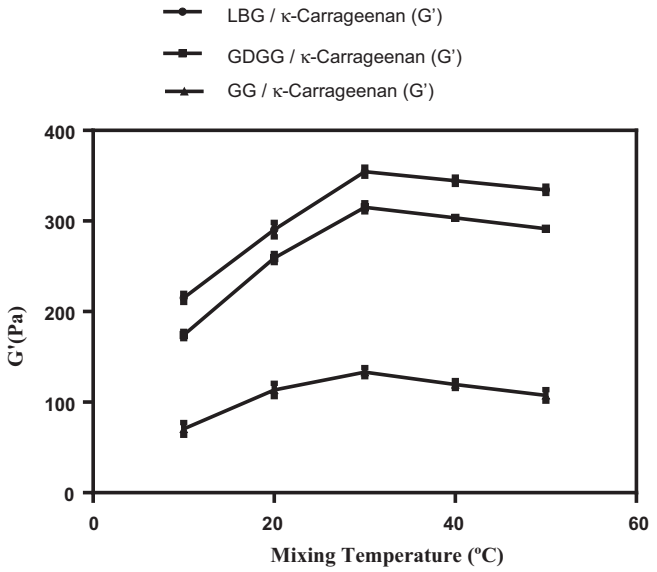

Fig. 4. Effect of mixing temperature on elastic modulus (G′) of galactomannan/κ-carrageenan blends.

4. Conclusion

Though, the catalytic activity of pepsin on GG is unusual and non-specific, it enables an alternative way to produce GDGG with significant reduction in galactose content and enhanced rheological characteristics. GDGG blend with κ-carrageenan exhibited a rheological behaviour similar to LBG blends. Also a two fold increase in the magnitude of elasticity compared to κ-carrageenan alone suggested a synergistic interaction with the formation of three dimensional networks. GDGG/κ-carrageenan blend also showed an improvement in the textural parameters similar to LBG/κ-carrageenan blend. Thus, modification of GG by (non-specific) unrelated enzymes to produce galactose-depleted guar galactomannans (GDGG) with enhanced rheological characteristics can be utilized in various food and non-food industries.

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