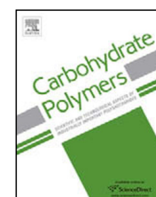
Carbohydrate Polymers 115 (2015) 253–259

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



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

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ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 13 August 2014
Accepted 13 August 2014
Available online 2 September 2014

Keywords:
Guar galactomannan
κ-Carrageenan
Pepsin
Locust bean gum
Synergy

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.

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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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http://dx.doi.org/10.1016/j.carbpol.2014.08.069
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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,

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

where η = intrinsic viscosity, K = 3.04 and α = 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.

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