Food Hydrocolloids 48 (2015) 149–154

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Comparative analysis of dietary fiber activities of enzymatic and gamma depolymerized guar gum

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ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form
29 January 2015
Accepted 9 February 2015
Available online 26 February 2015

Keywords:
Guar gum
Radiation depolymerization
Dietary fiber
Enzyme hydrolyzed

ABSTRACT

Gamma radiation depolymerized guar gum (RDGG) and partially enzymatically hydrolyzed guar gum (PHGG) were compared for their intrinsic viscosity (η), molecular weight distribution, proximate composition, mannose to galactose (M/G) ratio, glucose and bile acid dialysis retardation index (GDRI & BDRI) and production of short chain fatty acids (SCFA) during model intestinal fermentation. RDGG had a higher η value (37.90 ml/g) compared to PHGG (28.58 ml/g). PHGG had one peak with M_w of 12 kDa, while RDGG showed three peaks (M_w 1323.9 kDa, 614.02 kDa and 38.38 kDa) when subjected to gel permeation chromatography. Both RDGG and PHGG had similar proximate composition and M/G ratio. RDGG demonstrated higher GDRI and BDRI of 21.74% and 56.63% while PHGG had values of 12.74% and 0% respectively. Similar contents of SCFA were obtained using either RDGG or PHGG as carbon source. RDGG thus demonstrated improved physiological properties compared to enzyme hydrolyzed counterpart in *in vitro* assays.

1. Introduction

Dietary fibers are considered as an important nutritive component of human health and include wide variety of carbohydrates such as gums, pectin, lignin, cellulose, hemicellulose and resistant starch. Water soluble dietary fibers have received much attention in recent times due to their various physiological functions (Yoon, Chu, & Juneja, 2008).

Guar gum, one of the most promising soluble type of dietary fiber is a polygalactomannan derived from seeds of legume plant, *Cyamopsis tetragonalobus*. It is widely used as thickener in food products such as sauces, syrups, ice cream, instant foods, beverages, confectionaries and baked goods (Dogan, Kayacier, & Ic, 2007; Miyazawa & Funazukuri, 2006). Structurally, it is a galactomannan with a backbone of mannose units linked together by β-D-(1-4)-glycosidic linkage. Galactose units are linked to every alternate mannose units by α-1, 6 linkages on both sides of this backbone thus exhibiting a mannose to galactose ratio of 2:1 (Yoon et al., 2008). Molecular weight of guar gum is in range of 2000–3000 kDa and it provides extremely high viscosities in aqueous solutions even at low concentrations.

A WHO study group has recommended a daily intake of about 37 g total dietary fiber. The FASEB (Federation of American Societies for Experimental Biology) expert panel has recommended a daily intake of 20–35 g/day total dietary fiber from foods for the healthy, adult population of the USA (Burton-Freeman, 2000). Whereas, the American Diabetes Association has recommended a fiber intake of 40–50 g/day (American Diabetes Association, 1998). Guar gum in its native form is not suitable for use as a dietary fiber because it results in the liquid products with high viscosity when added to enteral formulas or liquid supplements at physiologically effective concentrations (Patrick, Gohman, Marx, DeLegge, & Greenberg, 1998). Moreover, high viscosity of guar gum is a limiting factor in its incorporation in foods at levels greater than 1 percent. Foods with physiologically relevant quantities of viscous fibers have very low consumer acceptability and have a slimy mouth feel and also cause tooth packing (Roberts, 2011). In addition, due to its high viscosity guar gum decreases the protein efficacy, lipid utilization and adsorption of nutrients by interfering with the digestion. It also results in slow gastric emptying (Yoon et al., 2008). Therefore, it needs to be depolymerized in order to be used as dietary fiber.

Physiological properties of guar gum can be improved by the controlled partial enzymatic hydrolysis by using β-endo-mannase

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http://dx.doi.org/10.1016/j.foodhyd.2015.02.013
0268-005X/

which is one of the most popular techniques. Endo-β-D-mannase hydrolyzes guar gum by selectively cutting mannose backbone-chain, leaving galactosyl groups intact. Partially hydrolyzed guar gum (PHGG) is one hundredth of original length of polymer and has an average molecular weight of 20 kDa. PHGG is GRAS (Generally recognized as safe) water soluble white powder which is odorless, tasteless, gives transparent solutions and is widely used as soluble type of dietary fiber since 1974 (Yoon et al., 2008). Its intake shows physiological effects such as increasing defecating frequency, reducing serum cholesterol and glucose concentration and production of short chain fatty acids (SCFA) resulting in lowering of pH of feces (Miyazawa & Funazukuri, 2006).

Use of gamma radiation could provide cheap and easy alternative to enzymatic hydrolysis of guar gum. There are numerous reports available in literature on γ-irradiation induced depolymerization of guar gum (Dogan et al., 2007; Gupta, Shah, Sanyal, Variyar, & Sharma, 2009; Jumel, Harding, & Mitchell, 1996). γ-irradiation can degrade guar gum by direct deposition of energy on polymer backbone or by hydroxyl (•OH) radical mediated reaction. Jumel et al. (1996) reported that molecular mass and viscosity of guar gum decreased with no significant changes in gross conformation during irradiation. However, radiation depolymerized guar gum (RDGG) has different molecular weight distribution from that of enzymatic hydrolyzed gum. This might lead to different physiological properties of radiation treated guar gum. To the best of our knowledge there are no reports on inter-comparison of gamma and enzymatic hydrolyzed guar gum for physiological functions. Use of bile acid dialysis retardation index (BDRI) and glucose dialysis retardation index (GDRI) to assess effect of dietary fiber on bile acids uptake in small intestine and jejunal nutrient absorption respectively was previously described by Adiotomre, Eastwood, Edwards, and Brydon (1990). Here, an attempt has been made to compare physiological functions of radiation and enzymatic hydrolyzed guar by different *in vitro* assays such as BDRI, GDRI and model intestinal fermentation.

2. Materials and methods

2.1. Materials

Guar gum sample of unknown molecular weight was obtained from Merck India Ltd. and partially enzymatically hydrolyzed guar gum (PHGG) (Sunfiber®) was provided by Taiyo Lucid Pvt. Ltd., Mumbai, India. H_2SO_4, acetonitrile and phenol were purchased from Merck India Ltd., India. $FeCl_3$, Na_2S, mannose, sodium taurocholate, $MnCl_2$, $CoCl_2$, soya trypticase broth and resazurine dye were procured from Himedia Lab Pvt. Ltd., India. Cysteine hydrochloride, $Ba(OH)_2$ and KH_2PO_4 were purchased from S.D. Fine-Chem Ltd., Mumbai, India. $MgSO_4$ and Na_2HPO_4 were obtained from Thomas Baker Ltd., Mumbai, India. Galactose and sodium azide were procured from BDH chemicals, India. $CaCl_2$ used was obtained from Loba-Chemie, Mumbai, India.

2.2. Preparation of samples for irradiation

Guar gum was irradiated in both powder and solution form. For irradiation in solution form gum solutions were made as 1% (w/v) in distilled water. Guar gum was dispersed in water using high speed mixer (Omni mixer, SORVALL, U.S.A.) for five minutes. The solutions were then kept overnight at 25 °C for complete hydration.

2.3. Gamma irradiation of samples

In powder form guar gum samples were subjected to radiation dose of 10, 15, 20, 25, 50 and 90 kGy while in solution form gum samples were irradiated at 2, 2.5, 3, 3.5, 4, 4.5 and 5 kGy. Irradiation was carried out at ambient temperature using a Co-60 gamma irradiator (GC-5000, BRIT, India). Dose rate as calculated by Fricke's dosimeter was 6.7 kGy/h with dose uniformity ratio of 1.13.

2.4. Purification of guar gum samples

Purification of guar gum was essentially carried out according to the procedure described earlier (Cunha, de Paula, & Feitosa, 2007). In brief, after irradiation in powder form guar gum was made to a 1% (w/v) aqueous solution and hydrated overnight. Samples irradiated in solution form were used as such. Solutions were centrifuged at 12800 g at 25 °C for 25 min. Twice the volume of distilled ethanol was added to the supernatant and the mixture was kept overnight for precipitation of polysaccharide. Solution was again centrifuged at 2050 *g* for 25 min at 25 °C. Purified guar gum was collected as pellet and was then freeze dried. Flakes obtained after drying were ground in pestle mortar and resulting free flowing white powder was stored in air tight bottles till further use.

2.5. Intrinsic viscosity

Intrinsic viscosity (η) of guar gum was calculated as per the procedure described earlier (Wang, Ellis, & Ross-Murphy, 2000). In brief, relative viscosity (η_r) was measured using a capillary viscometer from which specific viscosity (η_{sp}) was calculated:

$$\eta_{sp} = \eta_r - 1 \tag{1}$$

Further, η was determined from η_{sp} using Eq. (2):

$$\eta = \frac{\sqrt{1 + 1.4\eta sp} - 1}{0.7\ C} \tag{2}$$

Where, *C* is concentration of polymer solutions. All measurements were carried out at concentration of 0.1% w/v galactomannan.

2.6. Gel permeation chromatography (GPC)

GPC was carried out to calculate weight average molecular weight (M_w) for guar gum samples. Guar gum samples were analyzed by GPC column (BioBasic Sec-1000 column (300 mm × 7.8 mm 5 μm particle size) Thermo scientific) using HPLC (Ulitmate 3000, Dionex corporation) equipped with autosampler (Ultimate 3000 autosampler, Dionex Corporation, Germany) and refractive index detector (RH01, Shodex). Aqueous solutions of guar gum (0.2%) were injected (20 μl) using the autosampler and the data was acquired from the RI detector. Deionized water (Milli Q system, U.K.) was used as solvent system at a flow rate of 0.6 ml/min. Time vs. detector response data was exported into spreadsheet software (Excel 2007). Pullulan standards (10 kDa–25000 kDa, Fluka, U.S.A.) were also injected in similar conditions. Data of log (M_w, pullulan standards) vs. retention time (Rt) was plotted to obtain a straight line and a linear regression equation was calculated. Molecular weight of guar gum was calculated using linear regression equation obtained for pullulan standards and from the following equation with the Mark-Houwink-Sakurada constants reported for guar gum and pullulan (Miyazawa & Funazukuri, 2006).

$$M_g = 0.67M_p^{0.97} \tag{3}$$

Weight average molecular weight was calculated by following equation: