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Effect of enzymatic depolymerization on physicochemical and rheological properties of guar gum

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ABSTRACT

Depolymerization of guar gum using enzymatic hydrolysis was performed to obtain depolymerized guar gum having functional application as soluble dietary fiber. Enzymatic hydrolysis of guar gum significantly affected the physicochemical and rheological characteristics of guar gum. The depolymerized guar gum showed a significant increase in crystallinity index from 3.86% to 13.2% and flow behavior index from 0.31 to 1.7 as compared to native guar gum. Remarkable decrease in intrinsic viscosity and consistency index was also observed from 9 to 0.28 and 4.04 to 0.07, respectively. Results revealed that enzymatic hydrolysis of guar gum resulted in a polysaccharide with low degree of polymerization, viscosity and consistency which could make it useful for incorporation in food products as dietary fiber without affecting the rheology, consistency and texture of the products.

1. Introduction

Guar gum is a seed endosperm portion obtained from guar plant known as Cluster Bean. Guar seed was industrially processed into guar gum in 1940s and 1950s in United States (BeMiller, 2009). It is globally used as stabilizing and thickening agent in various industries such as food, pharmaceuticals, cosmetics, textile and oil recovery (Mudgil, Barak, & Khatkar, 2011b). In food industry, guar gum is utilized for its viscosity property in various food products such as sauces, soups, dairy products and baked food products (Mudgil, Barak, & Khatkar, 2011a). This high viscosity of guar gum is attributed to high molecular weight ranges between 0.1 and 2.8 million (Barth & Smith, 1981; Vijayendran & Bone, 1984). Guar gum molecule is composed of linear chain of β-1,4-linked mannose units with randomly attached α-1,6-linked galactose units. In last few decades, modification of guar gum is practiced due to its various applications. Particularly, depolymerization of guar gum is practiced for its nutritional value. Depolymerized guar gum is a low molecular weight galactomannan and is considered as a rich source of dietary fiber. When dispersed in water depolymerized guar gum give very low viscosity solution as compared to native guar. Recently, guar gum has been studied extensively for its health benefits (Butt, Shahzadi, Sharif, & Nasir, 2007). When guar gum (GG) is subjected to partial depolymerization its resultant product is known as partially hydrolyzed guar gum (Mudgil, Barak, & Khatkar, 2012c). Partially hydrolyzed guar gum (PHGG) is reported as a source of water soluble dietary fiber showing physiological benefits like increase in defecating frequency and reduction in serum cholesterol, free fatty acid and glucose concentration (Greenberg & Sellman, 1998).

Preparation of partially hydrolyzed guar gum by various methods has been widely studied. Partially hydrolyzed guar gum can be prepared using ultrasonication, enzymatic hydrolysis, free radical degradation, acid hydrolysis, irradiation and microwave techniques (Cheng, Brown, & Prud'homme, 2002; Jumel, Harding, & Mitchell, 1996; Reddy & Tammishetti, 2004; Shobha, Kumar, Tharanathan, Koka, & Gaonkar, 2005; Singh & Tiwari, 2009; Tayal & Khan, 2000; Wang, Ellis, & Ross-Murphy, 2000). Enzymatic depolymerization of guar gum is preferred using enzymes such as endo-β-D-mannanase (Yoon, Chu, & Juneja, 2008) and pectinase (Shobha et al., 2005). However, these are very expensive enzymes. Hence, in this study a cheap enzyme source was used for PHGG preparation. In the present study, an attempt has been made to study the effect of enzymatic hydrolysis (cellulase from *Aspergillus niger*) on physicochemical and rheological properties of partially hydrolyzed guar gum as compared to native guar gum.

2. Materials and methods

2.1. Materials

Guar gum used in this study was of commercial food grade and obtained from Hindustan Gums & Chemicals Ltd., India. Guar gum used in this study was fine particle size powder obtained after

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sieving through 200 mesh sieve. Guar gum sample was stored in refrigerator till used. Cellulase (*A. niger*) was obtained from USB Corporation, USA. Citric acid used in the study was obtained from Loba Chemie, India. All other chemicals used were from Sigma–Aldrich.

2.2. Enzymatic hydrolysis of guar gum

Guar gum was subjected to enzymatic hydrolysis using cellulase (*A. niger*) at concentration 0.19 mg/g, pH 5.6, temperature 50 °C and time 4 h (Mudgil, Barak, & Khatkar, 2012b). Firstly, pH of distilled water was maintained by addition of citric acid. After maintaining the required pH, cellulase was added at selected concentration. After adding enzyme, guar gum powder was sprinkled in vortex of distilled water using laboratory stirrer at 800 rpm. Controlled conditions for carrying out hydrolysis of guar gum were maintained using BOD shaking Incubator. During hydrolysis, guar gum solution was agitated at 100 rpm till 4 h of hydrolysis. After enzymatic treatment, a low viscous hydrolyzed guar gum solution was obtained. This resultant low viscous aqueous guar gum solution was sterilized for enzyme inactivation and then filtered and lyophilized to get partially hydrolyzed guar gum (PHGG) powder which was further subjected to physicochemical and rheological characterization.

2.3. Intrinsic viscosity

The intrinsic viscosity was estimated using an Ostwald's capillary viscometer at 25 °C in a concentration range of 0.02–0.08% for guar gum and 0.1–0.5% for partially hydrolyzed guar gum. All solutions were prepared by dispersing guar gum powder in vortex of water with lab stirrer at 800 rpm and mixed for 2 h. After mixing, solutions were rested for complete hydration for overnight at room temperature (25 °C). The complete hydrated solutions were then subjected to filtration via sintered crucible. The actual polysaccharide concentration in the solutions was determined by weighing the residue after total water evaporation in a hot air oven. Relative viscosity (η_r) was measured from ratio t/t_0, where t_0 is the flow time of a given volume of the solvent and t is the flow time of the same volume of gum solution respectively. Specific viscosity ($\eta_{sp} = \eta_r - 1$) was then calculated from relative viscosity. Specific viscosity (η_{sp}) was then used to derive reduced viscosity (η_r) using relationship $\eta_{red} = \eta_{sp}/C$. Intrinsic viscosity $[\eta]$ was then determined by calculating reduced viscosity at various concentrations in dilute solution and extrapolating to concentration $C = 0$. The intrinsic viscosity determination was performed in a waterbath at 25 °C. All flow time values were average of at least three replicates.

2.4. Degree of polymerization

Average molecular weight of guar gum and partially hydrolyzed guar gum was determined from intrinsic viscosity using Mark–Houwink's equation, $[\eta] = kM_v^{\alpha}$ with $\alpha = 0.732$ and $k = 3.8 \times 10^{-4}$ (Robinson, Ross-Murphy, & Morris, 1982). Average degree of polymerization (DP) for gum samples were determined by the following relationship:

$$\text{Average DP} = \frac{\text{molecular weight of polymer}}{\text{molecular weight of monomer}} \qquad (1)$$

Molecular weight of monomer used was 270 as reported in literature (Mahammad, Prud'homme, Roberts, & Khan, 2006).

2.5. Flow behavior index and consistency index

Flow behavior index (FBI) and consistency index (CI) of guar gum and partially hydrolyzed guar gum were analyzed determined using Brookfield viscometer. Aqueous solution of guar gum and partially hydrolyzed guar gum at 1% concentration (w/w) was prepared and left overnight for complete hydration. After overnight resting, gum solutions were subjected to viscosity estimation using spindle no. 1 (for PHGG) and spindle no. 4 (GG). Viscosity of GG was measured at 5–100 rpm with shear rates 2.6–52.1 s^{-1}. Whereas, viscosity of PHGG was measured at 20–100 rpm with shear rates 2.6–13.2 s^{-1}. Shear rates were calculated using Mitschka's equations (Mitscka, 1982). The experimental data obtained was fitted into Power Law model (Eq. (2)) to obtain flow behavior index (FBI) and consistency index (CI).

$$\sigma = K \cdot \gamma^n \qquad (2)$$

where σ is shear stress (N/m^2), γ is shear rate (s^{-1}), K is consistency index (Ns^n/m^2) and n (dimensionless) is flow behavior index (FBI).

2.6. Rheological analysis

Rheological analysis of guar gum and partially hydrolyzed guar gum at 1% concentration (w/w) were performed on controlled stress rheometer (Anton Paar, MCR 301). Cone plate geometry (cone angle = 1.01°, cone diameter = 25 mm) and concentric cylinders (outer cylinder diameter = 42 mm; inner cylinder = 39 mm) was used for guar gum and partially hydrolyzed guar gum, respectively. The effect of shear rate on viscosity of guar gum and partially hydrolyzed guar gum was evaluated. Flow curve for guar gum and partially hydrolyzed guar gum sample was also evaluated.

2.7. IR spectroscopy

Guar gum and partially hydrolyzed guar gum samples were studied by Fourier Transform Infrared (FTIR) Spectroscopy. Spectra were scanned between 4000 and 400 cm^{-1} on Shimadzu IR affinity-I 8000 FT-IR spectrometer under dry air at room temperature using KBr pellets supplied with FTIR unit. The gum samples were pressed directly on to attenuated reflectance KBr crystal into the sampling unit.

2.8. Crystallinity index

X-ray diffraction patterns of native guar gum and partially hydrolyzed guar gum were obtained using a Philips model XPert PRO diffractometer (anode Cu Kα, 45 kW, 40 mA) resolution of 0.02° at room temperature. Lyophilized samples of native and partially hydrolyzed guar gum were analyzed for their diffraction intensity between Bragg angles (2 theta) 10–80°. The empirical formula proposed by Segal et al. (1959) was used for estimation of crystallinity indices.

$$\text{Crystallinity index (\%)} = \left(\frac{1 - I_{18}}{I_{22.7}}\right) \times 100 \qquad (3)$$

where I_{18} is the intensity at 2 theta 18°, and $I_{22.7}$ is the intensity at 2 theta 22.7°.

3. Results and discussion

3.1. Intrinsic viscosity

Intrinsic viscosity of native guar gum and partially hydrolyzed guar gum are shown in Figs. 1 and 2. Enzymatic hydrolysis of native guar gum resulted in substantial decrease in intrinsic viscosity from 9 to 0.28 dL/g (Table 1). The enzymatic treatment leads to reduction in chainlength and molecular weight of guar gum (Mudgil, Barak, & Khatkar, 2012a; Mudgil et al., 2012c). That could be a reason for lower intrinsic viscosity of partially hydrolyzed guar gum.

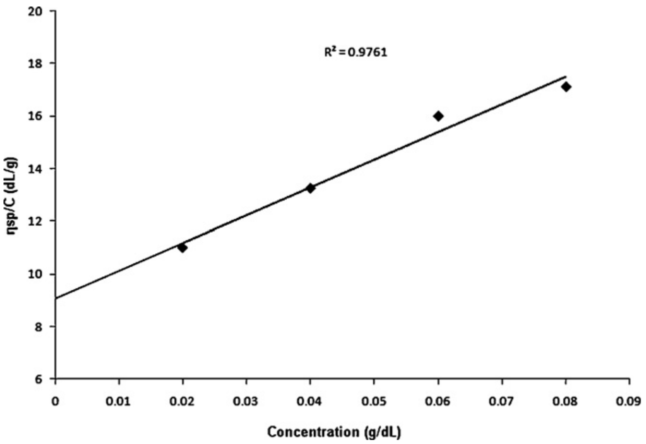

Fig. 1. Intrinsic viscosity plot for native guar gum.

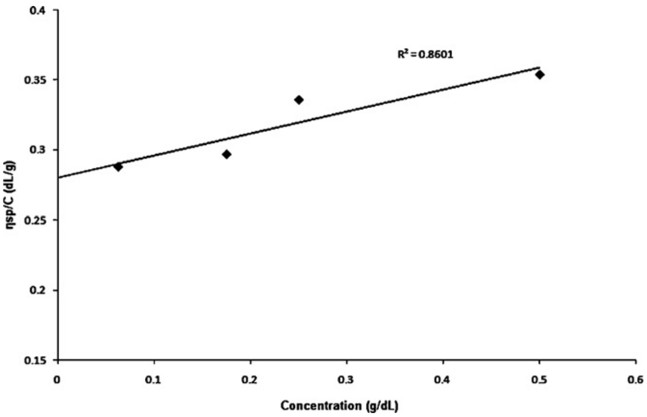

Fig. 2. Intrinsic viscosity plot for partially hydrolyzed guar gum.

3.2. *Degree of polymerization*

Degree of polymerization for native and partially hydrolyzed guar gum was determined as ratio of molecular weight of polymer and molecular weight of monomer. It was termed as average degree of polymerization because its estimation used viscosity average molecular weight. Enzymatic hydrolysis of guar gum showed marked decrease in average degree of polymerization as it involved breakage of bonds between mannose units and caused a decrease in chainlength of the polymer (Table 1). This decrease in chainlength decreased the molecular weight of the polymer and thus resulted in lower degree of polymerization of hydrolyzed guar gum.

3.3. *Flow behavior index and consistency index*

Guar gum solutions are pseudoplastic in nature (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). Flow behavior index (FBI) of guar gum and partially hydrolyzed guar gum was 0.3094 and 1.701, respectively (Table 1). FBI value below 1 represents the non-Newtonian behavior hence native guar showed shear thinning behavior which was also supported by rheological analysis of guar

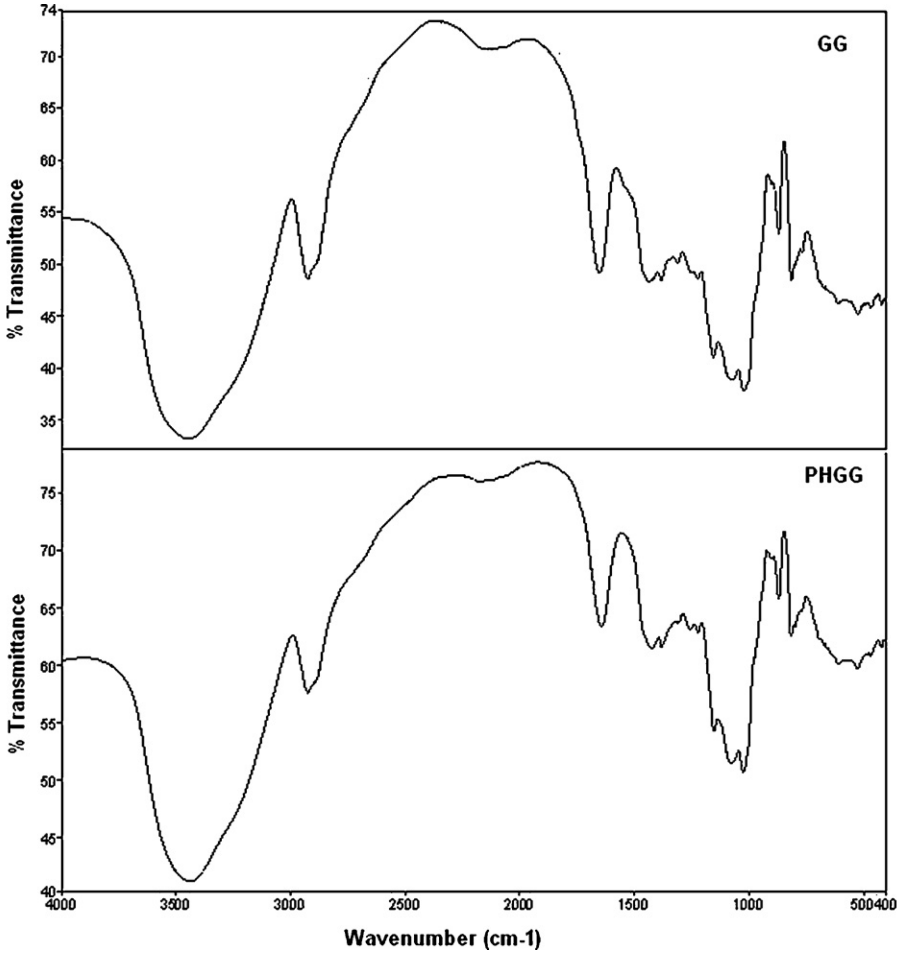

Fig. 3. IR spectra of native guar gum and partially hydrolyzed guar gum.

Table 1
Physicochemical analysis of native guar gum and partially hydrolyzed guar gum.

Characteristic	GG	PHGG
Crystallinity index	3.86	13.2
Flow behavior index	0.31	1.70
Consistency index	4.04	0.07
Intrinsic viscosity	9.0	0.28
Average degree of polymerization	3295.34	29.39

gum. Enzymatic treatment of guar gum resulted in tremendous increase in FBI value of guar gum probably due to depolymerization of the guar galactomannan. Results revealed that enzymatic hydrolysis of guar gum solution caused a change of the gum from non-Newtonian to Newtonian-like behavior due to depolymerization caused by enzymatic hydrolysis. Consistency index of guar gum and partially hydrolyzed guar gum was also analyzed to investigate the effect of enzymatic depolymerization on the consistency of guar gum. Consistency index of guar gum and partially hydrolyzed guar gum was 4.04 and 0.071, respectively. Enzymatic hydrolysis of guar gum showed a remarkable decrease in consistency index and increase in flow behavior index of partially hydrolyzed guar gum which justifies the use of partially hydrolyzed guar gum for dietary fiber supplementation of food products without affecting their consistency and texture. Dogan, Kayacier, and Ic (2007) also reported increase in FBI and decrease in consistency index of guar gum on depolymerization via irradiation.

3.4. *IR spectroscopy*

IR spectral analysis was carried to investigate any structural modification in guar gum caused by enzymatic depolymerization. IR spectra of enzymatically processed guar gum were almost superimposable with native guar gum (Fig. 3). FTIR spectra confirmed that there was no change in the functional group or the basic molecular structure of guar gum with partially hydrolyzed guar gum.

3.5. *Crystallinity index*

Crystallinity index is measure of percent crystallinity of a molecule and it tells about the alignment of molecules in particle structure. X-rays diffraction analysis revealed that native guar gum and PHGG showed amorphous structure. Crystallinity index (%) calculated for native guar gum and PHGG were 3.86% and 13.2%, respectively (Table 1). Enzymatic processing of guar gum resulted in increase in crystallinity of PHGG. This increase in crystallinity index of partially hydrolyzed guar gum may be due to lower degree of polymerization and chainlength of the molecule. Partially hydrolyzed guar gum molecules are more aligned than the native guar gum molecules in particle structure due to their shorter length and molecular size which is also justified by lower degree of polymerization.

3.6. *Rheological analysis*

Rheological behavior of 1% (w/w) aqueous solution of guar gum and partially hydrolyzed guar gum were analyzed at 25 °C. Figs. 4 and 5 show curve for shear stress–shear rate and viscosity–shear rate, respectively for guar gum solution. Shear stress–shear rate and viscosity–shear rate curve for partially hydrolyzed guar gum are shown in Figs. 6 and 7. Guar gum solution showed a pseudoplastic behavior. However, partially hydrolyzed guar gum showed a Newtonian like behavior. This pseudoplastic behavior of 1% native guar gum solution is also reported in the literature (Morris et al., 1981; Mudgil et al., 2011b). The pseudoplasticity of guar gum solution is concentration dependent and decreased with decrease in concentration. Pseudoplastic behavior of guar gum solutions almost changes to Newtonian behavior below 0.3% concentration (BeMiller & Whistler, 1993, chap. 8). Therefore, both the gums were expected to show pseudoplastic behavior but it was not happened due to Newtonian like behavior of partially hydrolyzed guar gum. The differences in the rheological behavior of native guar gum and PHGG at 1% (w/w) concentration may probably due to wide differences in their molecular weights and degree of polymerization. The molecular weight of native guar gum and PHGG are reported as 889,742 and 7936, respectively (Mudgil et al., 2012c). Guar gum showed shear thinning behavior because at higher shear rate unfolding of entanglement of the guar gum molecule may occur and caused a viscosity loss with increasing

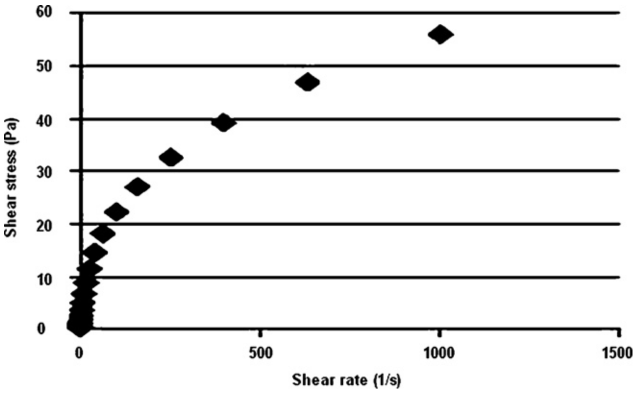

Fig. 4. Flow curve of native guar gum (1%, w/w) at 25 °C.

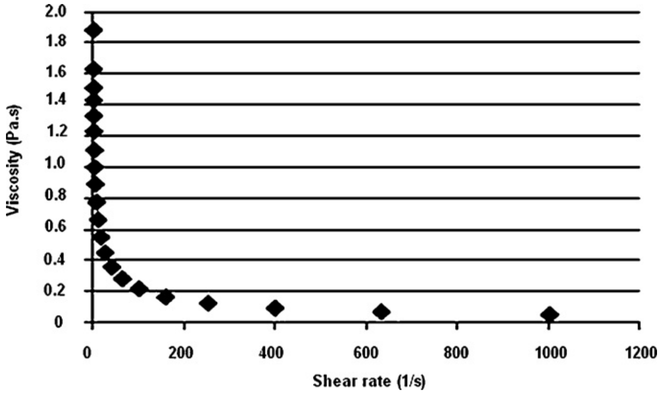

Fig. 5. Effect of shear rate on viscosity of native guar gum (1%, w/w) at 25 °C.

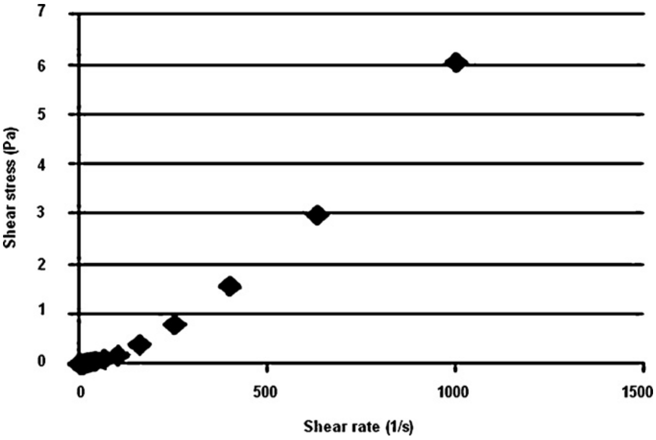

Fig. 6. Flow curve of partially hydrolyzed guar gum (1%, w/w) at 25 °C.

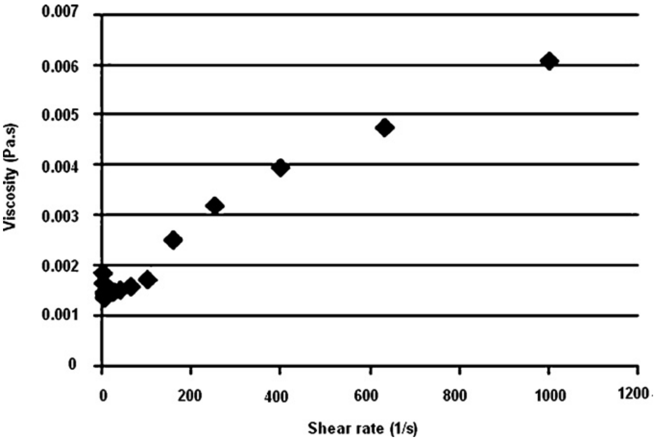

Fig. 7. Effect of shear rate on viscosity of partially hydrolyzed guar gum (1%, w/w) at 25 °C.

shear rate. The little or no effect of shear rate on viscosity of partially hydrolyzed guar gum may be justified with its low molecular weight and shorter chainlength.

4. Conclusions

Guar gum was hydrolyzed using cellulase (*A. niger*) to obtain depolymerized guar gum having commercial applications as dietary fiber. The depolymerized guar gum obtained after enzymatic hydrolysis was subjected to determination of intrinsic viscosity, flow behavior index, consistency index, degree of polymerization, rheological properties, crystallinity index. Enzymatic hydrolysis of guar gum significantly affected the physicochemical and rheological characteristics of guar gum. However, native guar gum and PHGG showed a similar basic structure. It can be concluded that the depolymerized guar gum may have the potential to be utilized as a functional food ingredient as soluble dietary fiber. Depolymerized guar gum may successfully be used as a source of soluble dietary fiber due to its functional properties such as low intrinsic viscosity, consistency index and similar basic molecular structure. Dietary fiber enriched food products can be prepared by supplementation of depolymerized guar gum as it will not affect the consistency and texture of the food products.

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