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Partially hydrolyzed guar gum as a potential prebiotic source

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ABSTRACT

Guar galactomannan was enzymatically hydrolyzed to obtain partially hydrolyzed guar gum which can be utilized as prebiotic source. In present study, growth of probiotics (Lactic Acid Bacteria strains) were studied with glucose, partially hydrolyzed guar gum and native guar gum. All the six strains were galactose &/or mannose positive using the API CHl 50 test. Almost all these strains showed an ability to assimilate partially hydrolyzed guar gum with respect to increase in optical density and viable cell count with concomitant decrease in the pH of the growth medium. *Streptococcus thermophilus* MD2 exhibited higher growth (7.78 log cfu/ml) while *P. parvulus* AI1 showed comparatively less growth (7.24 log cfu/ml) as compared to used lactobacillus and *Weissella* strains. Outcomes of the current study suggest that partially hydrolyzed guar can be considered as potential prebiotic compound that may further stimulate the growth of potentially probiotic bacteria or native gut microflora.

1. Introduction

Guar gum is natural biopolymer obtained from the seeds of *Cyamopsis tetragonolobus*. Native guar gum molecule is composed of linear backbone chain of β-1,4-linked mannose with side chain of α-1,6-linked galactose units [1]. Guar gum is obtained by separation of endosperm portion of seed from husk and germ portion. Endosperm portion of guar seed is chiefly galactomannan. Majority of guar gum is used for industrial and food applications. In food industry, guar gum is broadly used as stabilizer and thickener in various products such as juice, ice cream, sauce, ketchup, syrups, salad dressings etc. Food applications of guar gum are due to its very water binding capacity and texture management through water absorption. Apart from these food applications, guar gum has a very significant role in nutrition as dietary fiber. But unfortunately, it cannot be utilized as dietary fiber because of its very high viscosity in aqueous system [2]. In last few decades, guar gum is extensively researched for its health benefits, particularly, partially hydrolyzed guar gum which is prepared by enzymatic hydrolysis of guar gum [2,3]. Various enzymes such as mannanse, pectinase, cellulase etc. acts on the mannose-mannose linkage and reduce the back-bone chain of guar galactomannan and resulted in a product which is known as partially hydrolyzed guar gum. Partially hydrolyzed guar gum is a low molecular weight and low viscosity galactomannan [4]. Partially hydrolyzed guar gum cannot be digested by intestinal secretion and hence it comes in the category of dietary fiber [5].

Non-digestible oligosaccharides that can support the growth of health beneficiary microorganisms in the gut are termed as prebiotics [6]. Prebiotic oligosaccharides are non-cariogenic, non-digestible and low calorific compounds and are able to stimulate the growth and development of gastrointestinal microflora which are described as probiotic bacteria [7].

Lactic acid bacteria (LAB) have complex nutritional requirements and derive metabolic energy from homofermentative or heterofermentative carbohydrate fermentation [8]. Assimilation of oligosaccharide is essential for ecological fitness of LAB in most of their food-related and intestinal habitats [9,10]. Several non-digestible oligosaccharides have shown their growth stimulatory effects on lactic acid bacteria (LAB) and *Bifidobacterium* spp. [11–13]. Partially hydrolyzed guar gum can also be used as prebiotics as it contains oligosaccharides composed of galactose and mannose. Hence, present study was designed to investigate the effect of partially hydrolyzed guar gum on the growth of probiotic strains with respect to increase in the viable cell count which were measured by optical density and plating method.

2. Materials and methods

2.1. Materials

Commercial food grade guar gum used in this study was obtained from Hindustan Gums & Chemicals Ltd., India. Guar gum sample was passed through 200 mesh sieve to obtained fine particle size guar gum powder. This fine particle size guar gum sample was stored in refrigerator till further used for preparation of partially hydrolyzed guar gum and analysis. Cellulase (*Aspergillus 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.

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2.2. Bacterial strains and preservation

Total six strains of LAB, including four strains belonging to genus *Lactobacillus* (*L. helveticus* V3, *L. fermentum AI2*, *L. plantarum 86*, *W. cibaria 92*) and two strains of genus *Lactococcus* (*S. thermophilus* MD2, *P. parvulus* AI1) were used to study their prebiotic degradation attributes. All the strains were obtained from Dairy microbiology division, SMC College of Dairy Science, Anand, Gujarat state, India. Most of the isolates were isolated from traditional fermented foods including idli batter, dahi, cucumber, cabbage [14] while *L. helveticus* is established probiotic vaginal isolate [15]. Purified isolates were preserved at −20 °C on De Man, Rogosa and Sharpe (MRS, Hi-media, India) broth or M17 broth (Hi-media, India) containing 10% glycerol (v/v) and in freeze-dried form. The isolates were propagated twice before further use.

2.3. Enzymatic hydrolysis of guar gum

Guar gum was subjected to enzymatic hydrolysis using cellulase (*Aspergillus niger*) at concentration 0.19 mg/g, pH 5.6, temperature 50 °C and time 4 h [16]. Firstly, citric acid was added to distilled water to maintain its pH at 5.6. Cellulase enzyme was added to distilled water at selected concentration after maintaining the required pH via citric acid. Guar gum powder was sprinkled in vortex of distilled water with specific pH using laboratory stirrer at 800 rpm after addition of enzyme. Enzymatic hydrolysis of native guar gum was then carried out under controlled conditions maintained using BOD shaking incubator such as temperature, agitation and reaction time. Guar gum solution was agitated at 100 rpm till 4 h of hydrolysis. After completion of selected time for enzymatic hydrolysis, a very low viscosity (10 cps) hydrolyzed guar gum solution was obtained. This resultant low viscous aqueous guar gum solution was sterilized for enzyme inactivation (90 °C/15 min) and then filtered and subjected to freeze drying to obtain partially hydrolyzed guar gum (PHGG) powder which was further used to analyze its prebiotic potential.

2.4. Proximate analysis

Moisture content, fat content, protein content, ash content, total dietary fiber content, insoluble dietary fiber content, soluble dietary fiber content of unhydrolyzed and partially hydrolyzed guar gum were determined using AOAC standard methods of analysis [17].

2.5. Molecular weight & degree of polymerization

Average molecular weight of unhydrolyzed guar gum and partially hydrolyzed guar gum was determined from intrinsic viscosity values using Mark-Houwink's equation, $[\eta] = k M_v^{\alpha}$ with $\alpha = 0.732$ and $k = 3.8 \times 10^{-4}$ [18]. Average degree of polymerization (D.P.) for gum samples were determined as Average D.P. = molecular weight of polymer/molecular weight of monomer; where molecular weight of monomer used was 270 as previously reported in literature [19].

2.6. Apparent viscosity

Apparent viscosity of native guar gum and PHGG samples was measured using spindle-type rotational viscometer (Brookfield, U.S.A.). Firstly, the auto zeroing of viscometer was carried out in the air after fixing the specific spindle before measuring the apparent viscosity of each solution. Apparent viscosity of native guar gum solution and partially hydrolyzed solutions were measured at 20 rpm using spindle S62 and spindle S01, respectively. The selection of spindle for both the solutions was carried out as per the instructions given in manufacturer's manual. For viscosity analysis, solutions (1%) were prepared by dispersing 4 g of gum samples in 396 g of distilled water. Apparent viscosity of the samples was measured after 2 h of hydration at 20 °C. All measurements were done in triplicate at temperature of 20 °C.

2.7. Growth on prebiotic oligosaccharides

Initially all the strains were screened for their carbohydrate utilization using sugar fermentation strips (Hi-media, India). The strains were analyzed for growth on glucose and partially hydrolyzed guar gum. Growth with oligosaccharides present in partially hydrolyzed guar gum was observed for LAB strains by optical density measurements in tubes with concomitant decrease in pH. All the isolate were tested for fermentation in 5 ml of media with or without addition of respective prebiotic in 15 ml tubes to measure the change in OD_{620} values and pH within 24–48 h at 37 °C both in microaerobic condition. Samples were taken for OD and pH measurements, at 0 h, 24 h and 48 h of incubation, and respective dilutions of samples were plated on MRS agar and M17 agar media for the growth of lactobacillus and streptococci strains, respectively.

3. Results and discussion

3.1. Proximate analysis

Proximate composition and dietary fiber content (total, soluble and insoluble) of native unhydrolyzed guar gum and partially hydrolyzed guar gum are shown in Table 1. Unhydrolyzed guar gum showed higher moisture content as compared to partially hydrolyzed guar gum. This decrease in PHGG moisture content is due to the lyophilization or freeze drying operation involved in its preparation method. Lower value of protein was observed in case of partially hydrolyzed guar gum as compared to native guar gum which is due to insoluble nature of protein. During filtration step after enzymatic hydrolysis of native guar gum protein get separated from water soluble hydrolyzed guar gum portion hence lower protein value was observed in case of PHGG. Higher value of ash content in partially hydrolyzed guar gum was observed in comparison to unhydrolyzed guar gum which may be due to the neutralization step carried after enzymatic hydrolysis to neutralize citric acid which was added to maintain reaction pH during hydrolysis process. Total dietary fiber, insoluble dietary fiber and soluble dietary fiber content were not determined in native guar gum due to its very high viscosity whereas partially hydrolyzed guar gum showed a good amount of total dietary fiber as well as soluble dietary fiber. Partially hydrolyzed guar gum was shown to have 82.37% total dietary fiber and 79.52% soluble dietary fiber (Table 1). The results obtained for proximate analysis in this study are in agreement with the results reported in the literature [3].

3.2. Molecular weight & degree of polymerization

Intrinsic viscosity of unhydrolyzed guar gum and partially hydrolyzed guar gum were 8.7 and 0.32 dL/g, respectively. Reduced value of intrinsic viscosity was observed in case of partially hydrolyzed guar gum as compared to unhydrolyzed guar gum due to reduced molecular weight and chain length. The viscosity average molecular weight of native guar gum and partially hydrolyzed guar gum was 903,489.33 Da &

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

Parameters[a]	Guar gum[b]	PHGG[c]
Moisture (%)	11.12 ± 0.17	8.56 ± 0.19
Ash (%)	0.8 ± 0.02	2.41 ± 0.12
Protein (%)	4.51 ± 0.05	2.09 ± 0.36
Fat (%)	0.35 ± 0.04	1.16 ± 0.32
TDF (%)	–	82.37 ± 1.45
IDF (%)	–	2.85 ± 0.39
SDF (%)	–	79.52 ± 1.05

Note:
[a] The values are mean ± S.D. of determinations made in triplicates.
[b] Native guar gum.
[c] Partially hydrolyzed guar gum.