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Enzymatic production and characterization of manno-oligosaccharides from *Gleditsia sinensis* galactomannan gum

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ABSTRACT

Enzymatic hydrolysis of *Gleditsia sinensis* gum was performed to produce manno-oligosaccharides having functional applications as dietary fiber and prebiotics. The optimum hydrolysis conditions, including enzyme loading, temperature and time, from response surface methodology were 8.1 U/g, 57.4 °C and 34.1 h, respectively. The yield of DP 1–5 oligosaccharides was 75.9% (29.1 g/L). The Michaelis–Menten kinetics and molecular weight distribution were determined. The obtained oligosaccharides were further separated by HPLC and SEC, and the galactose distribution of *G. sinensis* gum was elucidated. Results indicated that *G. sinensis* gum has potential to produce value-added oligosaccharides in food industries.

1. Introduction

Galactomannan is a kind of natural and water-soluble polysaccharide consisting of a β-1,4-linked D-mannose backbone randomly substituted with single-unit α-1,6-linked D-galactose residues [1,2]. The functional properties of polysaccharide hydrocolloids are directly related with their structure. Polysaccharides could be depolymerized through various methods, such as γ-irradiation [3], ionizing radiation [4], microwave irradiation [5], chemical [6,7] and enzymatic hydrolysis [8–10]. In general, enzymatic hydrolysis can be kinetically controlled to produce desired end products. Degraded galactomannan with reduced molecular weight and viscosity could be applied as soluble dietary fiber in functional foods [11].

The susceptible enzymatic hydrolysis of galactomannan involves three types of enzymes: β-mannanase, β-mannosidase and α-galactosidase. Endo-1,4-β-D-mannanase (EC 3.2.1.78) randomly cleaves the β-1,4-D-mannosidic linkages within the main chain of mannan and mannan-type polysaccharides such as galactomannans and glucomannans to produce manno-oligosaccharides [12]. Only a few β-mannanases can release mannose in the hydrolysis of mannan [13]. The hydrolysis of galactomannan by β-mannanase is greatly affected by the extent and pattern of galactose substitution on the mannose backbone [14,15]. The β-mannanase is preferred to act on the region with low galactose substitution, and the liberated fractions mainly consist of non-substituted mannose residues [6,16].

Manno-oligosaccharides are non-digestible and could be potentially applied as dietary fiber and prebiotics [17]. Previous studies have confirmed the prebiotic activities of manno-oligosaccharides to human intestinal beneficial microflora (mainly bifidobacteria and lactobacilli) [18–20]. On the other hand, manno-oligosaccharides could prevent the fat storage through inhibiting the intestinal absorption of dietary fat in a high fat diet [21]. The researches with healthy adults as subjects revealed that the intake of 3.0 g manno-oligosaccharides per day could increase the amount of excreted fat and decrease the fat utilization [22–24]. Moreover, it was found that manno-oligosaccharides could suppress the elevation in blood pressure [21]. Manno-oligosaccharides have potential to be used as non-nutritional and functional food additives for selective growth of intestinal beneficial microflora, reduction of dietary fat absorption, and inhibition of the elevation of blood pressure.

Gleditsia sinensis Lam., a woody species (genus *Gleditsia*) in Leguminosae family, is widely distributed in China. Galactomannan as a storage polysaccharide exists in the endosperm of the *G. sinensis* seeds [25]. *G. sinensis* gum, similar to guar and locust bean gums both structurally and functionally, is a non-traditional source of industrial gums. At present, it usually served as thickener, stabilizer and flocculant in various industries of food, pharmaceuticals, petroleum drilling, paper and printing [26].

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This work aimed at the production of manno-oligosaccharides from *G. sinensis* gum by enzymatic hydrolysis. The optimum hydrolysis conditions were obtained using response surface methodology (RSM). The *G. sinensis* gum-derived oligosaccharides were further separated by size exclusion chromatography (SEC) and identified by high performance liquid chromatography (HPLC). The galactose substitution degree of *G. sinensis* galactomannan and the fractions with different degree of polymerization (DP) were determined to obtain galactose distribution information of *G. sinensis* galactomannan. Furthermore, the Michaelis–Menten kinetics and changes in apparent viscosity and molecular weight distribution (MWD) were investigated. The results of this study contribute to new applications of *G. sinensis* seed gum based on prebiotic activities, which are helpful to transform plant seed gum into high value-added oligosaccharides in food industry. The characterization of hydrolysis process and products can provide a better understanding of the *G. sinensis* galactomannan gum.

2. Materials and methods

2.1. Materials

Locust bean gum, guar gum and standard monosaccharides, including arabinose, mannose, galactose and glucose, were purchased from Sigma–Aldrich, Saint Quentin Fallavier, France. Standard manno-oligosaccharides of β-(1→4)-linked mannobiose (M2), mannotriose (M3), mannotetraose (M4), and mannopentaose (M5) were purchased from Megazyme International, Bray, Ireland. The endo-β-mannanase (EC 3.2.1.78), a commercial available enzyme purified from *Aspergillus niger*, was offered by Beijing Bosar Biotechnology Co., Ltd. (Beijing, China) and used after purification according to McCleary et al. [27] to remove the α-D-galactosidase, β-D-mannosidase, and exo-β-D-mannanase activities (<1 ppm). *G. sinensis* pods were kindly supplied by Shexian Forestry Bureau in Hebei, China. The seeds were manually separated and kept in a cool and dry place until further use. All the other chemicals and reagents used in this study were of analytical or higher grade.

2.2. Preparation of G. sinensis gum

The galactomannan gum from the *G. sinensis* seeds was prepared using the baking method [28,29]. Briefly, seeds (∼50 g) were baked in an oven at 120 ± 5 °C for 6 min. Then, the heated seeds were crushed in a laboratory muller for approximately 3 s. The halves of endosperm were sieved from the fragments of embryo and seed coat and ground to pass through a 0.125 mm sieve. The obtained yellowish powder was *G. sinensis* gum.

2.3. Enzyme assay and kinetics

The activity of β-mannanase was determined using locust bean gum as substrate. In brief, 0.5% (w/v) of gum solution was prepared in 0.1 M sodium acetate buffer (pH 6.0). The sample was incubated with the enzyme at 40 °C for 20 min, followed by addition of 3 volumes of anhydrous ethanol to terminate the reaction. The hydrolysate was then centrifuged at 4000 × *g* for 10 min. The enzyme activity was assayed based on the amount of reducing sugar in the supernatant using the 3,5-dinitrosalicylic (DNS) acid method [30,31]. One unit of β-mannanase activity is defined as the amount of enzyme required to release 1 μmol of reducing sugar equivalents per min.

G. sinensis gum is a kind of locust bean gum-like galactomannan, thus locust bean gum was used as a control in the enzyme kinetics determination. The enzyme kinetics was investigated by varying the substrate concentration in the range of 1–10 g/L. The Michaelis–Menten parameters, K_m and V_{max}, were evaluated from the Lineweaver–Burk plot of initial velocity versus substrate concentration. The catalytic constant (k_{cat}) was calculated as the ratio of V_{max} value to the initial enzyme concentration. The catalytic efficiency of β-mannanase to galactomannan substrate was assayed by the ratio of k_{cat}/K_m.

2.4. Oligosaccharide production

A typical enzymatic hydrolysis procedure was conducted by adding a certain amount of β-mannanase to the gum solution. A weighed amount of galactomannan gum was dispersed in the buffer solution under stirring. Then, the dispersion was placed in a boiling water bath for 10 min to inactive enzymes naturally existed in endosperm and accelerate gum hydration. After cooling at room temperature (25 ± 3 °C), enzyme was added and then mechanically stirred at a constant temperature to hydrolysis galactomannan. The optimum pH for enzymatic treatment was determined in 0.1 M sodium acetate buffer with pH range of 3.0–6.0. The effect of substrate concentration (2.0–8.0%) on the enzymatic hydrolysis was evaluated. In addition, the influence of enzyme loading, reaction temperature and time on the hydrolytic depolymerization were also investigated in the ranges of 5.0–9.0 U/g, 40–80 °C, and 0–48 h, respectively. All the determinations were done in triplicate.

2.5. Response surface experimental design

A central composite design (CCD) was applied to optimize the enzymatic hydrolysis conditions to further improve manno-oligosaccharide production from *G. sinensis* seed gum. Three independent variables, enzyme loading (x_1), temperature (x_2) and time (x_3), were studied at five levels (−α, −1, 0, +1, +α), resulting in a design of 20 experiments. Analysis of variance (ANOVA) and response surfaces were applied using Design Expert software 8.0.6 to evaluate the effects of the factors (individual or combined) on oligosaccharide production. The obtained data were fitted with a second-order polynomial equation by a multiple regression technique. Models and regression coefficients were considered significant by computing the *F*-value at a probability (p) of 0.05, 0.01 or 0.001.

It was found in present research that the DP > 5 fractions took a small proportion of the *G. sinensis* gum-derived products and were very difficult to be separated. Moreover, it has been reported that hydrolysis of locust bean gum with β-mannanases resulted in the production of DP 1–6 oligomers, mainly DP 3 and DP 4 fractions [32,33]. Therefore, this study used the production of DP 1–5 fractions to evaluate the hydrolytic efficiency of the enzyme. The yield of DP 1–5 oligosaccharides (Y) was used as the response in RSM model.

2.6. Characterization of the manno-oligosaccharide production

2.6.1. Apparent viscosity measurement

Enzymatic hydrolysis of *G. sinensis* gum with the aid of β-mannanase was performed on the basis of the optimum conditions obtained from RSM. During the initial 30 min of the enzymatic hydrolysis, the apparent viscosity variations of the gum solution were monitored by an LVDV-III Ultra Rheometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) with a constant shear rate of 10 s^{-1}.

2.6.2. MWD measurement

During the enzymatic hydrolysis, changes in MWD of *G. sinensis* and locust bean gums were analyzed by gel permeation chromatography (GPC) equipped with three columns in series, TSK PW_{XL} Guard Column, TSKgel G6000 PW_{XL} and TSKgel G3000PW_{XL} (TosoHaas). The mobile phase was 0.2 M phosphate buffer at pH 6.8, with