Research Article Product Composition Analysis and Process Research of Oligosaccharides Produced from Enzymatic Hydrolysis of High-Concentration Konjac Flour

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ABSTRACT: There is a huge variability in reducing sugars, viscosity, and composition of oligosaccharides in the hydrolyzed products of konjac flour with different concentrations. We analyzed the factors affecting reducing sugars, viscosity, and the average degree of polymerization (DP) during the preparation of oligosaccharides from konjac flour hydrolyzed by β-mannanase under the high-concentration solute hydrolysis model. Hydrolysate of konjac flour, using concentrations ranging from 50 to 200 g/L, was directly added into 20 U/mL of β-mannanase solution. The results showed that when the proportion of the water content in the solution decreased, the viscosity of the solution and the DP of polysaccharides changed significantly. When the viscosity of the hydrolysate was controlled within the range of 30−20 mPa·s, the concentration of the reducing sugars was maintained in the range of 9−13 g/L and the average DP of the polysaccharides was controlled in the range of 2.42−9.78. We also found that a high concentration of hydrolysate was beneficial for decreasing the production of reducing sugars, and the diversification of macromolecular glycan was beneficial to the preparation of functional sugars. Moreover, we observed that the proportion of reducing sugars with free water content was high and that the preparation of oligosaccharides via the high-concentration solid-state method increased product diversity.

INTRODUCTION

Konjac oligosaccharides are a mixture of small-molecule polymers containing 2−20 sugar rings, which are linear micromolecular glycans with D-mannose and D-glucose linked through β-1,4-glycosidic bonds. Konjac oligosaccharides are an essential class of functional oligosaccharides, are used to treat constipation, suppress cancer cells, and improve gut microbes. Konjac oligosaccharides are prepared from konjac flour, and the primary treatment methods are often used in enzymatic hydrolysis, acid hydrolysis, and microwave deposition. Konjac flour is a polysaccharide; its aqueous solution has high-viscosity characteristics, enzyme protein has been adsorbed easily, and the activity of glycoside hydrolase would be weakened. Therefore, for the enzymatic hydrolysis, konjac flour with a concentration of less than 50 g/L is used to form konjac gum after swelling, which is followed by the addition of hemicellulase for the degradation of glucomannan of konjac flour. Because the konjac flour used for hydrolysis is of low solute concentration, a large amount of reducing aldose is produced during the hydrolysis process, and the average degree of polymerization (DP) of the polysaccharide is low. The finished product is a combination of monosaccharides with 2–4 sugar rings. The low-concentration liquid enzymatic hydrolysis treatment method was used in many previous studies. However, such a method is less efficient and is not conducive to industrial preparation. Therefore, in...
enzymatic hydrolysis of the high-concentration konjac solution, konjac flour has difficulties in swelling, such that the water-soluble gum substance becomes solid without fluidity.\textsuperscript{21−23} This type of hydrolysis leads to a small contact surface of the enzyme and a low hydrolysis rate. Besides, the yield of the enzymatic hydrolysate is influenced by the high viscosity of the konjac gum, which is not conducive to the catalysis of mannanase.

In the process of enzymatic hydrolysis, the traditional method, konjac flour was first prepared in an aqueous solution, and then the enzyme solution was added. Moreover, in this study, we used the reverse method and directly added konjac flour to the acidic mannanase solution to form a semisolid viscous shape, which was further hydrolyzed at 60 °C to form a concentrated hydrolysate. In this paper, the hydrolysis and product composition of konjac flour were analyzed in a high-concentration hydrolysis model.

### RESULTS AND ANALYSIS

**Analysis of the Viscosity of Konjac Flour and Konjac Flour Hydrolysate.** Konjac flour was dissolved in water to form a high-viscosity konjac flour solution, which had poor fluidity and was considered a non-Newtonian type of fluid.\textsuperscript{24} Once the konjac flour was under high-concentration conditions in the reaction tank, a high-viscosity solution was obtained. The sediment was muddy and without fluidity, and it was difficult to stir. If a lot of manganase protein is absorbed by konjac flour and can not thoroughly mixed, the affinity of the enzyme to the substrate is significantly weakened, leading to low catalytic efficiency and weak hydrolysis reaction, which is not conducive to the enzymatic hydrolysis process. The design of the hydrolysis process can be facilitated by understanding how the viscosity of konjac raw materials influences the fluidity analysis. Figure 1A shows the viscosity curve of konjac flour as different concentrations of konjac flour were dissolved in water and measured by a viscometer. When the low-concentration low quantity of konjac flour was dissolved into a water solution, the viscosity of solution was low and was easy to stir.

![Viscosity curves of konjac gum and hydrolysate](https://dx.doi.org/10.1021/acsomega.9b04218)
Besides, with increasing konjac flour concentration, the viscosity of the solution would also increase, showing a positive correlation. Thus, the viscosity of the konjac gum at a concentration of 20 g/L was higher than 100,000 mPa·s, and no fluidity was observed.

During the traditional process of enzymatic hydrolysis, konjac gum is formed by preformulation, and then the enzyme solution is added for enzymatic hydrolysis. This traditional method is only suitable with a low concentration of the substrate. Konjac easily cross-links with metal ions under alkaline conditions. It forms a gel-like precipitate, which is not conducive to the binding of the active site of the enzyme, resulting in degradation difficulty. Several studies have reported using a low concentration of konjac flour to form konjac gum via swelling, followed by hydrolysis.20,25,26 The finished products have a high hydrolysis percentage, long consumption time, and a single product.

To improve the hydrolysis efficiency and concentration of hydrolyzed products, we adopted the enzymatic hydrolysis technology of the high-concentration substrate. By adding konjac flour to the enzyme solution, β-mannanase cleaved the β-1,4-glycosidic bonds of glucomannan in konjac gum to form small-molecule glycans and reduce the viscosity of polysaccharides. Figure 1B–E shows the change in viscosity during the β-mannanase-induced hydrolysis of konjac flour (at concentrations of 50, 100, 150, and 200 g/L). The concentrations of konjac flour in Figure 1B,C were 50 and 100 g/L, respectively. The unit enzyme protein degradation of konjac powder was less (bearing ratio η small), a high hydrolysis rate was obtained, and the viscosity decreased rapidly—the viscosity decreased to 3.5–9 mPa·s within 15 min. At a concentration of 150–200 g/L, the hydrolysis viscosity was controlled below 30 mPa·s, and the hydrolysis reaction took longer than 60 min (Figure 1D,E). We found that the time required for the viscosity to reach 15 mPa·s was much lower than that required for the enzymatic hydrolysis to produce small-molecule monosaccharides. When the viscosity decreased to a certain threshold, the viscosity changed very slowly as the reaction time increased, but the concentration of monosaccharides increased. Therefore, timely control of viscosity is conducive to the control of monosaccharide production.

The process of combining konjac flour with the enzyme solution for hydrolysis and dissolution was analyzed. As shown in Figure 1F–I, 2 kg of konjac flour (200 g/L) was added to 10 L of enzyme solution (konjac flour concentration 200 g/L), and the swelling rate of the konjac flour was higher than the enzymatic hydrolysis rate. The konjac flour swelled and quickly became solid and viscous (Figure 1F). The enzyme protein was encapsulated in the water-swollen konjac flour. After 5 min, the konjac flour became semisolid, the amount of free water increased, the crude fiber began to peel off, and the soluble sugars separated (Figure 1G); after 30 min of enzymatic hydrolysis, the semisolid konjac flour was degraded into a liquid state, which could be agitated and had a certain fluidity (Figure 1H); over time, the change in viscosity became minimal. The viscosity after 60 min did not change substantially; it remained within the range of 60–30 mPa·s (Figure 1I). Thus, in the hydrolysis process of high-concentration konjac flour, a small amount of konjac flour can be added several times to maintain the hydrolysis viscosity in a low-viscosity state, which is conducive to equipment stirring.

Figure 2. Analysis of konjac flour hydrolysate. (A) Control of viscosity at 30 mPa·s; analysis of variation of difference in different concentrations of konjac flour releasing reducing sugars by enzymatic hydrolysis. (B) Changes in the content of reducing sugar in the hydrolysate of konjac flour of different concentrations. The results showed that the reducing sugar of certain concentrations can inhibit the further catalysis of the enzyme. (C) Relationship between reducing sugars, DP, and reaction rate. (D) Yield rate of reducing sugars yield rate in different concentrations of konjac flour.

To improve the hydrolysis efficiency and concentration of hydrolyzed products, we adopted the enzymatic hydrolysis technology of the high-concentration substrate. By adding konjac flour to the enzyme solution, β-mannanase cleaved the β-1,4-glycosidic bonds of glucomannan in konjac gum to form small-molecule glycans and reduce the viscosity of polysaccharides. Figure 1B–E shows the change in viscosity during the β-mannanase-induced hydrolysis of konjac flour (at concentrations of 50, 100, 150, and 200 g/L). The concentrations of konjac flour in Figure 1B,C were 50 and 100 g/L, respectively. The unit enzyme protein degradation of konjac powder was less (bearing ratio η small), a high hydrolysis rate was obtained, and the viscosity decreased rapidly—the viscosity decreased to 3.5–9 mPa·s within 15 min. At a concentration of 150–200 g/L, the hydrolysis viscosity was controlled below 30 mPa·s, and the hydrolysis reaction took longer than 60 min (Figure 1D,E). We found that the time required for the viscosity to reach 15 mPa·s was much lower than that required for the enzymatic hydrolysis to produce small-molecule monosaccharides. When the viscosity decreased to a certain threshold, the viscosity changed very slowly as the reaction time increased, but the concentration of monosaccharides increased. Therefore, timely control of viscosity is conducive to the control of monosaccharide production.

The process of combining konjac flour with the enzyme solution for hydrolysis and dissolution was analyzed. As shown in Figure 1F–I, 2 kg of konjac flour (200 g/L) was added to 10 L of enzyme solution (konjac flour concentration 200 g/L), and the swelling rate of the konjac flour was higher than the enzymatic hydrolysis rate. The konjac flour swelled and quickly became solid and viscous (Figure 1F). The enzyme protein was encapsulated in the water-swollen konjac flour. After 5 min, the konjac flour became semisolid, the amount of free water increased, the crude fiber began to peel off, and the soluble sugars separated (Figure 1G); after 30 min of enzymatic hydrolysis, the semisolid konjac flour was degraded into a liquid state, which could be agitated and had a certain fluidity (Figure 1H); over time, the change in viscosity became minimal. The viscosity after 60 min did not change substantially; it remained within the range of 60–30 mPa·s (Figure 1I). Thus, in the hydrolysis process of high-concentration konjac flour, a small amount of konjac flour can be added several times to maintain the hydrolysis viscosity in a low-viscosity state, which is conducive to equipment stirring.
Correlation Analysis of Reducing Sugars and Average DP during Enzymatic Hydrolysis. The hydrolysis of konjac flour was accompanied by the production of reducing sugars, which are mostly monosaccharides, and higher concentrations of monosaccharides affect the yield of oligosaccharides. The concentration of reducing sugars reflected the degree of hydrolysis of konjac flour, the rate of hydrolysis, and the average DP of the polysaccharides. In this paper, the enzymatic hydrolysis process was carried out at 60°C. β-mannanase cleaved the β-1,4-linked glycosidic bonds, which was accompanied by the production of a large amount of D-glucose, D-mannose, and aldose with reducing ends. In Figure 2A, the hydrolysis viscosity was controlled at 30 mPa·s; reducing sugars occurred more in the hydrolysis products of konjac flour at low concentrations but less in the hydrolysis products of konjac flour at high concentrations. By measuring the reducing sugar in the solution of each concentration (Figure 2B), we found that the concentration of reducing sugars formed by hydrolysis with different concentrations of konjac flour was maintained within the range of 9–13 g/L. When converted into reducing sugars per gram of raw materials, the number of reduced monosaccharides released per gram of konjac flour by high-concentration hydrolysis was only 70 mg. Still, the monosaccharides released by the low-concentration konjac flour hydrolysis solution could reach 227 mg/(g raw materials).

As shown in Figure 2C, in the hydrolysis system with 200 g/L of konjac flour, the enzyme activity in the solution was 20 U/mL. When adding konjac flour to form a concentration of 50 g/L, because per unit enzyme protein bearing konjac flour was 1.25 mg, the hydrolysis process was quickly completed in a short time. Each gram of raw material could release 282 mg of reducing sugars, and the average DP was 2.41. In the 200 g/L high-concentration konjac flour hydrolysate, the per unit enzyme protein bearing konjac flour was 5 mg, the reducing sugars produced was only 70 mg/(g raw material), and the average DP of hydrolysis was 9.78.

When konjac flour was added to the enzyme solution, the solvent water was involved in the adsorption reaction of konjac flour. As the amount of konjac flour increased, the hydrolysis solution changed from the diluted state to the concentrated state and semisolid state. Thus, the high-concentration konjac aqueous solution, with a decreased amount of water, influenced the catalytic ability of the enzyme, thereby inhibiting the degradation rate of reducing sugars (Figure 2D).

Therefore, when konjac flour was added to the enzyme solution, the 1 U enzyme bearing the weight of konjac flour was less, and the hydrolysis process was quickly completed. As a result, a large amount of reducing sugar has been produced. With the increase in konjac flour, the enzyme protein load increased, and hydrolysis was incomplete, more so in the macromolecular material cleavage stage, producing less reducing sugar. Therefore, by artificially controlling the viscosity of the hydrolysate at 30 mPa·s, we were able to maintain the reducing sugar content at a concentration of 9–13 g/L. Moreover, by controlling the corresponding enzyme activity in the raw material, the catalytic direction of the enzyme was able to be regulated, and the ratio of the reducing sugar formed could be effectively controlled.

Figure 3. Matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF-MS) analysis and composition of konjac flour hydrolysate. (A) Primary structure mass spectrum under the concentration of 50 g/L; (B) primary structure mass spectrum under the concentration of 100 g/L; (C) primary structure mass spectrum under the concentration of 200 g/L; and (D) konjac oligosaccharide composition of konjac flour hydrolysate.
Table 1. Molecular Weight Comparison of Konjac Oligosaccharide Products

| Product | Molecular Mass | Debris Peak [M + K]^+ m/z | 50 g/L Konjac Flour Hydrolysate | 100 g/L Konjac Flour Hydrolysate | 200 g/L Konjac Flour Hydrolysate |
|---------|----------------|----------------------------|---------------------------------|---------------------------------|---------------------------------|
| DP-1    | 180.2          | 219.01                     | ✓                               | ✓                                | ✓                                |
| DP-2    | 342            | 381                        | ✓                               | ✓                                | ✓                                |
| DP-3    | 504.08         | 543.08                     | ✓                               | ✓                                | ✓                                |
| DP-4    | 666.23         | 705.23                     | ✓                               | ✓                                | ✓                                |
| DP-5    | 828.41         | 867.41                     | ✓                               | ✓                                | ✓                                |
| DP-6    | 990.51         | 1039.52                    | ✓                               | ✓                                | ✓                                |
| DP-7    | 1152.77        | 1191.77                    | ✓                               | ✓                                | ✓                                |
| DP-8    | 1314.96        | 1353.96                    | ✓                               | ✓                                | ✓                                |
| DP-9    | 1477.15        | 1516.15                    | ✓                               | ✓                                | ✓                                |
| DP-10   | 1639.36        | 1678.36                    | ✓                               | ✓                                | ✓                                |
| DP-11   | 1801.6         | 1840.6                     | ✓                               | ✓                                | ✓                                |
| DP-12   | 1963.8         | 2003                       | ✓                               | ✓                                | ✓                                |
| DP-13   | 2125.7         | 2207                       | ✓                               | ✓                                | ✓                                |
| DP-14   | 2288           | 2369                       | ✓                               | ✓                                | ✓                                |
| DP-15   | 2450.3         | 2531                       | ✓                               | ✓                                | ✓                                |
| DP-16   | 2613.5         | 2694                       | ✓                               | ✓                                | ✓                                |
| DP-17   | 2774.7         | 2856                       | ✓                               | ✓                                | ✓                                |
| DP-18   | 2937.0         | 3018                       | ✓                               | ✓                                | ✓                                |
| DP-19   | 3099           | 3180                       | ✓                               | ✓                                | ✓                                |
| DP-20   | 3261           | 3342                       | ✓                               | ✓                                | ✓                                |
| DP-21   | 3423.2         | 3504                       | ✓                               | ✓                                | ✓                                |

Mass Spectrometry (MS) Analysis of the Hydrolysate. Figure 3A–C shows the composition of the enzymatic hydrolysate of konjac flour at concentrations of 50, 100, and 200 g/L. Small-molecule glycan products were identified according to the molecular fragment peak, and hydrolysis products formed at different concentrations were searched according to the multiple of 162 Da differences between each sugar molecule; results are shown in Table 1. It can be seen from Table 1 that few types of hydrolytic products were formed in 50 g/L konjac flour, whereas more types of hydrolytic products were formed when the concentration was more significant than 100 g/L. The types and percentages of oligosaccharides were analyzed according to the peak area ratio of the primary structure mass spectra of the konjac products in Figure 3. In Figure 3D, in the hydrolysate with a low concentration of konjac flour (50 g/L), the formed products were mainly mannose, DP-2 (disaccharide), and DP-3 (trisaccharide) because of excessive hydrolysis. According to the statistical calculation of the peak area of each sugar molecule, it was found that disaccharides were 65% of the formed product; in the hydrolysate with a high concentration of 100 g/L konjac flour, the oligosaccharides were mainly DP-2 (disaccharides) to DP-5 (pentasaccharides), and the proportion of DP-3 (trisaccharides) was the highest (21%). The oligosaccharide formed from the hydrolysate of 200 g/L konjac flour showed two gradient hydrolysis processes. The products in the first gradient were DP-2 to DP-10 glycoside polymers with a content of 46.8%, and the proportion of DP-3 (trisaccharides) was 15%. The products in the second gradient were DP-11 to DP-18 with a content of 53.2%, of which DP-11 accounted for 20%.

**DISCUSSION**

In this study, we analyzed the relationship between reducing sugars, viscosity, and DP during the process of preparing oligosaccharides from konjac flour hydrolyzed by acidic β-mannanase. A reverse addition method was adopted in this study, and as such, an enzyme solution with a specific concentration was pre-prepared, and then konjac flour was added to the enzyme solution. In the early stage, because of excessive enzymes and rapid hydrolysis, the hydrolysis rate was higher than the swelling rate of the konjac flour, and the konjac flour was dissolved into the enzyme solution and hydrolyzed continuously. The macromolecular substance gradually became a small molecular substance. As shown in the following enzymatic hydrolysis phase, the reaction time increased, glucomannan was gradually hydrolyzed into sugar monosaccharides of different molecular weights. During the entire reaction process, a large amount of water was needed for the chemical reaction. During the swelling phase of konjac flour, water was also needed, and micromolecular glucomannan had been formed through hydrogen bonding and van der Waals forces.

Konjac flour was added to the reaction system containing the enzyme solution for the dissolution and hydrolysis of konjac flour. The reaction system used in the experiment was in an acidic environment of pH 5.5, and konjac flour was added to the enzyme solution, resulting in a solution concentration of 200 g/L of konjac flour. The swelling rate of konjac flour was still higher than the enzymatic rate, and konjac flour swelled and rapidly turned into wet flour (Figure 1D). Although the acidic environment reduced the swelling ability of the konjac glucomannan,27 the concentration of konjac flour was too high, resulting in a solid viscous state. The enzyme was encapsulated in the konjac flour swollen with water and became semisolid after 5 min. The amount of free water increased, the crude fiber began to peel off, and soluble sugars were separated (Figure 1E). After 30 min of enzymatic hydrolysis, the semisolid konjac flour was degraded into a liquid state, which could be agitated and had a certain fluidity (Figure 1F). Over time, there was a minimal change in viscosity, which was maintained within the range of 20–30 mPa·s.

Considering the water content of the reaction system, the weight of the solids in the reaction system was 20% (w/v), and the free water in the reaction system was relatively small, which
was not conducive to the migration of free enzymes. Besides, the process of the konjac flour absorbing water to form konjac flour involved the combination of physical and chemical processes. Numerous water molecules were required for consumption. The chemical process of the combination of konjac flour and water was destroyed by the enzymatic hydrolysis method, resulting in the release of a large number of water molecules, which were then involved in the reaction of small molecular sugars, thus destroying the powerful absorbing properties of konjac flour toward the water. It was also found that the amount of reducing sugars with free-water content was high. Therefore, the preparation of oligosaccharides by a high-concentration solid-state method was beneficial to the diversity of products and the reduction of the proportion of monosaccharides.

While studying the enzymatic preparation of konjac oligosaccharides, we demonstrated that the amount of reducing aldose formed by excessive enzymes was high, and the DP value was low. As the concentration of the substrate increased, the concentration of the solute increased, the bearing ratio \( \eta \) of the enzyme solution also increased, and the hydrolysis rate decreased. The hydrolysis process, in which macromolecules were cleaved to form small molecular substances, required the involvement of plenty of free water in the enzyme reaction. Once the concentration of free water decreased, it would affect the hydrolysis rate of small molecular substances being further degraded into monosaccharides, and the proportion of monosaccharides formed was not high. As shown in Figures 2D and 3, a 50 g/L konjac flour solution, the concentration of solute formed was 5%, whereas the concentration of solvent water was 95%, which is beneficial to the catalysis function of the enzymes and can maintain the activity of the glycoside hydrolase. During the process of high-concentration enzymatic hydrolysis, the combination of konjac flour and water formed konjac gum, and water could bind to small-molecule sugars formed by the hydrolysis of glycans as a ligand. Once free water was not available, the catalytic activity of the enzyme was altered, limiting further cleavage of glycosidic bonds, and the proportion of macromolecular substances was high.

Therefore, to obtain a high concentration of konjac oligosaccharides and reduce the monosaccharides in the production process, the viscosity of enzymatic hydrolysate must be controlled. A high-concentration solute is conducive to reducing free water in the reaction system, effectively controlling DP, and high-quality konjac oligosaccharides can be obtained.

**EXPERIMENTAL SECTION**

**Materials.** First-level konjac flour was purchased from Yuyuan Food Corporation (Lijiang, Kunming, China). \( \beta \)-Mannanase (8000 ± 400 U/g), with a protein molecular weight of 44–62 kDa and an isoelectric point of 0.75, was purchased from Qactive Biotechnology (Kunming, Yunnan, China). 3,5-Dinitrosalicylic acid (DNS) was obtained from Lanji Technology Development (Shanghai, China), and phenol was obtained from Fengchuan Chemical Reagent Technology (Tianjin, China). All other reagents were produced in China and were of analytical grade purity.

**Determination of \( \beta \)-Mannanase Activity.** The konjac gum substrate (5 g/L) was prepared using a 0.2 M dipotassium hydrogen phosphate–citric acid buffer solution (pH 5.5). Konjac gum substrate (1.8 mL) was added into two sample tubes and one blank tube and preheated in a 60 °C water bath for 5 min. The diluted \( \beta \)-mannanase solution (0.2 mL) was then added to the sample tubes. After 30 min, 3 mL of DNS reagent \(^{28} \) was added to each tube. Additionally, a diluted \( \beta \)-mannanase solution (0.2 mL) was added to the blank tube. All tubes were placed in boiling water for 5 min and cooled, and then, 10 mL of distilled water was added. After mixing, the reaction liquid was colorized on a spectrophotometer with a colorimetric wavelength of 540 nm. The enzymatic activity of the solution was extrapolated from a standard curve of reducing sugar. An enzyme unit (U) was defined as the amount of enzyme required to hydrolyze konjac gum (5 g/L) to produce 1 \( \mu \)mol of mannose at pH 5.5 and 60 °C.

**Determination of Reducing Sugar Products.** Konjac gum was hydrolyzed by acidic \( \beta \)-mannanase, and the hydrolyzed oligosaccharide product was appropriately diluted 10-fold with distilled water. The diluted oligosaccharide solution (0.2 mL) was added into a test tube, and 3 mL of DNS reagent was added. The mixture was placed in a boiling water bath for 5 min. Then, water was added to bring the reaction volume to 15 mL. Colorimetric analysis was performed to calculate the reducing sugar content \(^{29} \) in the enzymatic hydrolysate. We used the following equation to calculate the percentage of conversion of reducing sugars:

\[
\text{Conversion of reducing sugar (\%)} = \frac{\text{amount of reducing sugar in the enzymatic hydrolysate} - \text{amount of free reducing sugar}}{\text{amount of konjac flour} \times 100}
\]

**Determination of the Average DP of Enzymatic Hydrolysate.** The average DP refers to the ratio of the total sugar content \(^{30} \) of konjac flour to the amount of reducing sugar in the enzymatic hydrolysate. The closer the value is to 1, the more thorough the hydrolysis is. We used the following equation to calculate the average DP:

\[
\text{Average DP} = \frac{\text{total sugar content of konjac gum}}{\text{amount of reducing sugar in the enzymatic hydrolysate}}
\]

**Effect of Viscosity on the Fluidity of Konjac Flour and Konjac Hydrolysate.** Konjac flour (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 g) was added to 100 mL of 60 °C water and mixed evenly with a magnetic stir bar. After 1 h, the solution was swollen and mixed uniformly, and then, the solution was cooled to room temperature (25 °C). The viscosity was measured using a rotor viscometer (NDJ-8S, Shanghai LICHEN-BX Technology Co., Ltd., China). Rotor no. 1 was required to determine the low viscosity, and rotor no. 4 was required to determine the high viscosity. Maintaining the temperature of the konjac gum solution at 25 °C, the rotor was put into the konjac gum solution. The drive device enabled the rotor to rotate steadily for 3 min and scale range to reach 50%, and the viscosity value was read.

Next, 1.25 g of \( \beta \)-mannanase flour was added into 500 mL of 60 °C hot water and mixed evenly; the \( \beta \)-mannanase activity in the solution was 20 U/mL. Four triangular flasks were placed in a water bath, each bottle was filled with 125 mL of the enzyme solution, and the temperature was kept at 60 °C for 5 min. Konjac flour (5, 10, 15, and 20 g) was added to the enzyme solution. The solutions were magnetically mixed to form 50, 100, 150, and 200 g/L konjac flour solutions. The
viscosity of the hydrolysis solution was monitored every 5 min. The reducing sugar content was determined when the viscosity of each concentration gradient was between 15 and 30 mPa·s. The experiment was repeated three times to obtain the experimental average.

**Hydrolytic Product Analysis by MALDI-TOF-MS.** The concentrated sample of konjac oligosaccharides was diluted fivefold, and the total soluble sugar concentration was controlled at 40 g/L. The diluted sample and 2,5-dihydroxybenzoic acid matrix solution were mixed on the MALDI plate at a 1:1 ratio and then dried at room temperature. The composition of oligosaccharides was analyzed using a MALDI TOF mass spectrometer. The instrument was ionized by MALDI, and a total of 10,000–20,000 laser emissions were obtained for each mass spectrum. The mass spectrum was collected by the hyperspectral MALD-TOF (Bruker Daltonics, Inc.) mass spectrometer in cation mode.

**Statistical Analysis.** Results are shown as the means of three biological replicates, and the error bars indicate the standard deviation. The statistical significance of the results was evaluated using Student’s t-test. Correlation analysis was performed using Spearman correlation analysis (SPSS 19.0). Origin 8.5 software was used for preparing graphs and data processing.

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**Notes**

The authors declare no competing financial interest.

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