Intrinsic Viscosity and Reducing Sugar Profiles of Degraded Glucomannan using Cellulase

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Abstract. High viscosity of glucomannan has been limit its applications. Hence, the long polysaccharide glucomannan which consists of β-linked glucose and mannose needs to be degraded to broaden its utilisations. Enzymatic degradation is known as a safe method for food material treatment. Specifically targeting at 1,4-β-D-glycosidic linkages, cellulase has potential as a cleavage for glucomannan. This work aimed to study performance of cellulase in degrading glucomannan. The change in intrinsic viscosity and reducing sugar during degradation was observed.Degradation of 1% glucomannan by 20 ppm cellulase for 300 min decrease d the viscosity from 12,420 to 353 cps. This degradation was also confirmed by the increase of reducing sugar amount from 1,238.57 to 8,510.00 µg/ml. This work showed the success of glucomannan degradation by cellulase.

1. Introduction

Extracted from natural source, glucomannan of Amorphophallus sp. has various properties. Tatirat & Charoenrein extracted the konjac glucomannan and obtained its viscosity of 30 Ps at 1% concentration [1]. The high viscosity of glucomannan has an advantage for encapsulating active agents by gelation method but limits the use of spray drying method [2]. As spray dryer required 300 cP viscosity of the feed solution [3], glucomannan matrix solution needs to be degraded to avoid nozzle blockage. Decreasing the length of glucomannan chain has been studied as a method for producing low-viscosity glucomannan [4]. Biological degradation using enzymes was known as a relatively safe method for degradation, especially for food products as no toxic substance generated [5]. Chen et al hydrolized konjac glucomannan using β-mannase enzyme and reduced its molecular weight to 3,089.81 cP [6]. Other than β-mannase, cellulase enzyme had previously studied as a degradation agent of glucomannan. Yang et al successfully decreased the apparent viscosity of glucomannan up to 200 mPa.s using cellulase enzyme [7]. Previous study of Wardhani et al showed that cellulase use on enzymatically degraded glucomannan solution supported the feed preparation of spray drying encapsulation process [8]. However, the viscosity decrease of glucomannan solution has not been investigated.
In this study, the glucomannan was hydrolyzed using cellulase enzyme from Aspergillus niger. The objective of this study was to investigate the effect of glucomannan and cellulase concentration on the reduction of intrinsic viscosity of glucomannan solution. The resulted solution was further studied for its reducing sugar concentration.

2. Materials and Methods

2.1. Materials
Purified glucomannan was obtained from Amorphophallus konjac of Now Food brand. Cellulase from Aspergillus niger (≥0.3 U/mg) was bought from Sigma Aldrich. Other supporting materials used were in pro analyze specifications.

2.2. Glucomannan degradation
Enzymatic degradation of glucomannan was conducted in room temperature. Distilled water (1000 mL) was placed in a beaker glass, followed by the addition of glucomannan powder and cellulase enzyme with various concentrations. The degradation process was carried out for 300 min with propeller mixing using Hightech IKA RW 20 Digital Mixer.

2.3 Intrinsic viscosity determination
Cannon-Fenske viscometer was used to determine the intrinsic viscosity of glucomannan solution by preparing solutions in different concentrations (0.01, 0.025, 0.05, 0.075, and 0.1 g/L). The flow duration of each glucomannan solution \( t_{\text{sample}} \) at a certain concentration \( c \) and water as the solvent \( t_{\text{solvent}} \) were recorded for the calculation of specific viscosity \( \eta_{sp} \) and reduced viscosity \( \eta_r \) using Equation (1) and (2), respectively.

\[
\eta_{sp} = \frac{t_{\text{sample}} - t_{\text{solvent}}}{t_{\text{solvent}}} \\
\eta_r = \frac{\eta_{sp}}{c}
\]

The intrinsic viscosity of the glucomannan was obtained from the average value of intercepts of specific viscosity and reduced viscosity plotted with the concentration.

2.4 Direct reducing sugar (DRS) measurement
The reducing sugar was determined using 3,5-Dinitrosalicylic acid (DNS) method [9]. Sample (1 mL) was added by DNS reactant (3 mL) and heated in boiling waterbath for 5 min. The sample was cooled down in room temperature and checked for its absorbance by spectrophotometer at 550 nm wavelength. The reducing sugar concentration was obtained by plotting the absorbance on glucose standard curve.

3. Results and Discussion
Glucomannan composed of glucose and mannose monomers linked by β-1.4-glycosidic bond [10]. The bond could be hydrolyzed using cellulase and produced glucomannan oligosaccharides and monosaccharides [11]. Glucomannan was enzymatically degraded using cellulase for 300 min at room temperature with constant stirring. The degradation study was conducted by the variation of cellulase concentrations (0 – 20 ppm) in 1% glucomannan solution and glucomannan concentrations (0.5 – 1.75% w/v) using 15 ppm cellulase. The degradation caused by cellulase was studied for the intrinsic viscosity reduction, as well as the change of reducing sugar as the supporting data.

For 1% w/v glucomannan solution, the enzymatic degradation lowered the intrinsic viscosity of glucomannan solution for 2.865 ml/g using 20 ppm of cellulase (Figure 1). Addition of cellulase decreased the final intrinsic viscosity. Increasing concentration of cellulase on glucomannan hydrolysis intensified the cellulase contact on glucomannan chain, which resulted on producing more oligosaccharides/monosaccharides.
The use of higher concentration of glucomannan solution was not recommended as the less degradation effect observed. Higher concentration of glucomannan solution had a higher intrinsic viscosity value after 300 min degradation using 15 ppm cellulase. Higher glucomannan concentration formed viscous solution that interrupted the molecular movement of the enzyme. This suggested that the enzyme concentration was too low to deal with high glucomannan concentration. This result was similar to previous study of Wardhani et al on enzymatically alginate degradation [12].

![Graph showing intrinsic viscosity of glucomannan after enzymatic degradation](image)

**Figure 1.** Intrinsic viscosity of glucomannan after enzymatic degradation

Cleavage of the β-glycoside bond of glucomannan by the cellulase produced glucose and mannose, which could be determined as the amount of reducing sugar [13]. Figure 2 shows that the higher cellulase concentration increased the reducing sugar produced from the degradation. A steep increase of reducing sugar level detected after 45 min of degradation using 20 ppm of cellulase and followed by relative constant curve at 8.510 µg/ml.

On the other hand, the use of 15 ppm of cellulase on higher glucomannan concentration shown insignificant degradation performance (Figure 3). The highest rise of reducing sugar was obtained by degrading 0.5% glucomannan solution in that cellulase concentration. Nevertheless, the similar increase rates of reducing sugar were shown in glucomannan solution with the concentration more than 1%. The slower degradation rate in high glucomannan concentration was also found by Li et al [14]. The results of reducing sugar amount supported by the previous viscosity result, in which the high amount of reducing sugar was indicated by the lower viscosity of glucomannan solution.
Figure 2. Development of DRS values as the effect of cellulase concentration

Figure 3. Development of DRS values as the effect of glucomannan concentration

4. Conclusion
Cellulase use as enzymatic degradation agent successfully decrease the glucomannan viscosity. The degradation lowered the viscosity up to 2.042 mL/g by performing degradation on the solution contained 20 ppm cellulase. This high concentration of cellulase also impacted on the increasing amount of reducing sugar produced by degradation process. To obtain the maximum effect of glucomannan degradation, the use of high cellulase concentration on low concentration of glucomannan solution was preferable.

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