Effect of KOH as Deacetylation Agent on Physicochemical Properties of Glucomannan

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Abstract. Glucomannan is one of the high viscosity polysaccharides with high water absorbing capacity as well as solubility. These properties limit glucomannan applications. Hence the property needs to be modified. Glucomannan has 5-10% acetyl groups which conferred its physicochemical properties. Replacing the acetyl with alkali, transforms the physicochemical characterisation of the glucomannan. This study aimed to determine the effect of deacetylation conditions on the physicochemical properties of glucomannan of Amorphophallus oncophyllus. The effects of concentration of KOH (0.1, 0.25 and 0.5 M) and reaction time (2, 8, 16 and 24 h) on the degree of deacetylation (DD), solubility, and viscosity of the glucomannan were studied. The concentration of KOH and reaction time showed a positive impact on the DD of the glucomannan. Solubility and viscosity decreased with increasing KOH concentration and extending the duration of the deacetylation. It was suggested that the changes in the physicochemical properties were due to the replacement of the acetyl group with the alkali during deacetylation. However, the effect of this replacement was not linear with the variables. This nonlinearity could be due to the various removal distributions of the acetyl groups during the deacetylation.

1. Introduction

Glucomannan, the hemicellulose derivation, is one of the most important chemical components present in the porang bulb (Amorphophallus oncophyllus). It comprises of D-glucopyranose and D-mannopyranose in ratio 1.6-2:1, and connected with β-1,4 glycosidic linkage. Glucomannan has a relatively high molecular weight (200,000 - 2,000,000 Daltons) with size between 0.5 - 2 mm which is 10-20 times larger than starch cells [1,2,3].

Recently, glucomannan has been paid increasing attention as encapsulation excipient and controlled release material since it is non-toxic, safe, hydrophilic and biodegradable [4]. Glucomannan has a relatively high absorption index of 100 grams of water/gram. It contains a few acetyl groups at position C-6, in every 9-19 sugar units [5]. The presence of the acetyl groups in glucomannan restrained the formation of both intramolecular hydrogen bonds and intermolecular hydrogen bonds between glucomannan molecules, so that the acetyl group responsible for its solubility in water [6].
Elimination of the acetyl groups produces deacetylated glucomannan, which can build junction zones through hydrogen bonding, dipole-dipole (among water molecules and OH groups of deacetylated glucomannan), van der Waals, charge transfer and hydrophobic interactions etc. 

It has been reported that deacetylation of glucomannan using alkaline solution forms a gel structure. As the deacetylation reaction proceeds in the presence of alkali, the acetyl groups are partially removed from the glucomannan backbone, thus yielding deacetylated segments, which are responsible for gel formation. The replacement of alkali group results in reducing water solubility of glucomannan due to strongly diminishes steric hindrance caused by the acetyl groups. The increasing amount of alkaline solution showed a positive effect on the deacetylation. Thus the removal of all acetyl groups on glucomannan may occur when using an excess alkali solution.

Some alkalis have been reported as deacetylation agent for glucomannan in alcohol medium including NaOH, KOH, and Ca(OH)2. KOH shows better performance as deacetylation agent than NaOH in mechano-chemical assisted deacetylation. Increasing concentration of deacetylation agent results in decreasing viscosity. However, the mechano-chemical assisted reaction is not common in deacetylation process. Hence, the aim of this work was to study the effect of deacetylation conditions on physicochemical properties of glucomannan of A. oncophyllus. Effects of concentration of KOH (0.1, 0.25 and 0.5M) and reaction time (2, 8, 16 and 24 h) were studied on degree of deacetylation, solubility, and viscosity of deacetylated glucomannan.

2. Materials and methods

2.1. Materials

Glucomannan was isolated from A. oncophyllus flour (80-100 mesh) using Rahayu et al. KOH p.a (99%), ethanol (96%), HCl p.a, PP indicator were obtained from Merck.

2.2. Deacetylation of Glucomannan

The deacetylation was conducted based on the method of Chen et al. Ethanol (50%, 100 ml) and glucomannan (5 g) were added to beaker glass and stirred at 250 rpm for 30 min at 50°C. A particular concentration of KOH was added to the mixture and kept stirring for corresponding times. The solid which was separated using filter paper was oven-dried at 40°C to obtain deacetylated glucomannan (DGM).

2.3. Degree of deacetylation (DD)

Determination of degree of deacetylation was conducted following the method of Zhang et al. DGM (5 g) was suspended in 50 ml ethanol (75%) at 50°C for 30 min. KOH solution (0.5 M, 5 ml) was added and kept stirred for 48 h. The suspension was titrated using HCl 0.1 M and used phenolphthalein as indicator. The DD was calculated as:

\[
DD = \frac{\omega_o - \omega}{\omega_o} \times 100\%
\]

where \(\omega_o\) and \(\omega\) are the amounts of acetyl in the untreated glucomannan and that in the treated glucomannan, respectively. The \(\omega_o\) is calculated
where $V_2$ and $V_0$ are a volume of HCl for blank titration and that for untreated glucomannan before acetylation, respectively. $N_{HCl}$ is a concentration of HCl for titrant, $M_{acetyl} = 43$ g/mol and $m$ is the sample to be titrated. The $\omega$ is calculated:

$$\omega = \frac{(V_2-V_0) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100\%$$  \hspace{1cm} (3)

where $V_2$ and $V_1$ are a volume of HCl for blank titration and that for deacetylated glucomannan, respectively. $N_{HCl}$ is a concentration of HCl for titrating, $M_{acetyl} = 43$ g/mol and $m_s$ is sample mass to be titrated.

2.4. Solubility

Solubility of samples was determined based on the method of Akpa et al. [14]. Deacetylated glucomannan (0.1 g) diluted with 10 ml distilled water which was heated at 60°C for 30 min in waterbath. After centrifuged at 4000 rpm for 20 min, the supernatant was collected and preweight before being dried.

$$\text{Solubility (\%)} = \frac{W_{\text{dried suspended solid}}}{W_{\text{Supernatant}}} \times 100\%$$  \hspace{1cm} (4)

2.5. Viscosity of deacetylated glucomannan

DGM (0.5 g) and 50 ml of distilled water were stirred magnetically for 30 min. Viscosity was measured every 10 min for three measurements using Viscosimeter Cannon Fenske. Distilled water is used for a reference liquid. The time that it took for the liquid to pass between two etched marks is measured. The viscosity is calculated:

$$\eta_x = \rho_x \frac{t_x}{t_a} \eta_a \times 100\%$$  \hspace{1cm} (5)

where $\eta_x$ and $\eta_a$ are the viscosity of sample (cP) and reference (cP); $\rho_x$ and $\rho_a$ are the density of sample (g/ml) and reference (g/ml); $t_x$ and $t_a$ are time (s) from upper to lower marks for sample and references, respectively.

3. Results and Discussions

3.1. Degree of Deacetylation

Effects of KOH concentration and reaction time deacetylation on the DD of glucomannan is presented in Figure 1. This figure shows KOH concentration was in line with the DD. The more acetyl groups of glucomannan replaces when an alkali solution are available.. Li et al. [15] reported higher alkali concentration is better for removing the acetyl of glucomannan. The result supported that deacetylation is a hydrolysis reaction which affected by the concentration of the alkali [6].
Increasing concentration of KOH from 0.1 to 0.5 M resulted in increasing the DD from 39.87% to 84.50% at 24 h. This result suggested that 0.5 M of KOH was not enough to remove all the acetyl groups. The removal of all of the acetyl groups on glucomannan occurs when used an excess of alkali solution applied [9,15]. Maekaji [9] showed that 1 gram of glucomannan require $3.45 \times 10^{-4}$ mol OH- to remove the acetyl groups.

Figure 1 also showed a positive correlation between duration of deacetylation time and the DD. Extended the reaction time allowed a longer contact between the acetylts and the alkali which increased the probability of the substitution. The same result is reported by Pan et al. [11] who found the concentration and prolonged treatment would be necessary for increasing the deacetylation of glucomannan.

3.2. Solubility

Figure 2 shows solubility of deacetylated glucomannan decreased with increasing KOH concentration and reaction time of deacetylation. Increasing KOH from 0.1-0.5 M resulted in decreasing solubility from 72.34- 79.19% after 24 h. Glucomannan contains 5-10% of acetyl groups which reported to confer the aqueous solubility of glucomannan. This result supported that the acetylts are involved in enhancing hydrogen bonding with water and reduce hydrophobic interaction [16]. The decrease of solubility could be due to loss of acetyl groups during deacetylation. The alkaline solution serves to reduce the solubility properties glucomannan in the process of deacetylation as acetyl group at glucomannan will be separated with the aid of an alkaline substance, and then glucomannan will bind hydrogen with the OH group and forming a gel [17].
This result was supported by Prasetya et al. [18] in which the greater the alkali concentration drove lower solubility of deacetylated glucomannan. Herranz et al. [7] stated that the addition of alkali significantly reduces the steric barriers caused by the acetyl groups. This leads to the formation of non-covalent crosslinks such as hydrogen bonds in glucomannan. Hydrogen bonding and hydrophobic interaction, in particular, are considered the main reasons responsible for gel formation. Also, increasing the pH facilitated the formation of the anionic group of glucomannan chain that can change the function of water structures in the tissues and modify the end properties of the glucomannan gel [15]. The deacetylated glucomannan molecule hydrates and forms hydrogen bonds between the O atoms in water and the OH group in the glucomannan chain [19]. The acetyl group in the glucomannan chain will be released due to the presence of hydrogen bonds resulting in agglomeration. Clumping in the form of this gel will cause the solubility of glucomannan to decrease [17].

3.3. Viscosity

Effects of KOH concentration and reaction time deacetylation on a viscosity of deacetylated glucomannan is presented in Figure 3. In general, viscosity decreased with extended the reaction time. Pan et al. [11] reported the prolong of deacetylation time reducing the viscosity. Increasing deacetylation time affects the formation of an increasing gel. Takigami et al. [20] indicated that as a gelling agent, glucomannan has a unique ability to form reversible gels and irreversible gels under different conditions. This gel formation is caused by the substitution of the acetyl group with the OH-group. The acetyl group on glucomannan will more and more react with the OH group at KOH so that the breaking process of CH$_3$-CO group. The viscosity is affected by the molecular weight in which the larger molecular weight acetyl group (50 g/mole) has been substituted by the OH group with a molecular weight of 17 g/mole. As a result, the viscosity of glucomannan will also decrease. Wang et al. [21] showed a positive linear relationship between the intrinsic viscosity log value and the log value of the molecular weight. Another reason is that deacetylation of glucomannan occurs in an
alkaline solution, resulting in water solubility and self-aggregation decreasing. Thus the extent of molecular chain expansion becomes smaller [19].

Figure 3. Effect of deacetylation time and KOH concentration on viscosity

4. Conclusions

Replacing the acetyl by using KOH transformed the physicochemical characteristics of deacetylated glucomannan. These results suggested that concentration of KOH and reaction time affected the DD, solubility, and viscosity of deacetylated glucomannan differently. The DD increased with increasing concentration of KOH and extending the duration of deacetylation. Meanwhile, KOH concentration and reaction time were in reverse relation with solubility and viscosity. These changes in the physicochemical properties could be due to the replacement of the acetyl group with the alkali during deacetylation. However, the effect of this replacement was not linear with the variables. This nonlinearity could be due to the various removal distributions of the acetyl groups during the deacetylation.

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