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Simultaneous Effect Of Temperature And Time Of Deacetylation On Physicochemical Properties Of Glucomannan

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The presence of acetyl group in the backbone of water-soluble glucomannan is responsible for its solubility. This solubility requires being modified to support glucomannan application as an encapsulant. Removing the group by deacetylation reduces the solubility. This work was aims to study simultaneous effect of temperature and time of deacetylation on glucomannan physicochemical properties. The deacetylation was conducted in ethanol using Na_2CO_3 at various times (2, 4, 8, 16, 24 and 28 h) and temperatures (room temperature, 40, 50, and 60°C). The deacetylated samples were subject to degree of deacetylation (DD) as well as solubility and swelling analysis in pH 1.2 and 6.8. DD was in positive correlation with deacetylation time and temperature. The solubility of the deacetylated glucomannan at both pHs decreased along with the deacetylation time. A reverse trend was found for swelling determination at both pHs. Increasing deacetylation temperature showed a positive impact in swelling determination but not occuring on the solubility. Interestingly, the swelling and solubility were lower at pH 1.2 than those at pH 6.8. These results showed physicochemical of deacetylated glucomannan was pH sensitive, hence have a potency as an excipient of controlled-release drug delivery system.

Keywords: deacetylation, Na_2CO_3, glucomannan, solubility, swelling

INTRODUCTION

Glucomannan is a non-ionic linear heteropolysacharides consist of D-glucopyranose and D-mannopyranose. Acetyl groups are attached at C-6 position on average every 9-19 sugar units (Takigami 2000). This hydrophilic and biodegradable compound is known as one of the highest viscosity copolymers and commonly used as functional food material. It has an extraordinary water binding capacity and able to absorb over 100 times of its dry weight (Herranz et al. 2013).

The ability of glucomannan to form gel and its high viscosity has been an interest for application in foods and pharmaceutical areas including controlled release excipient (Jin et al. 2014). However, its high water-

solubility has been limiting its application as the excipient. The acetyl groups are responsible for its solubility, hence inhibit its gelation and coating ability (Gao and Nishinari 2004).

The presence of the acetyl group restricts formation of hydrogen bond intra and inter glucomannan molecules (Wardhani et al. 2016). In order to improve the glucomannan performance as the controlled drug release, the acetyl needs to be removed by using alkali. This modification transforms the ability to build junction zones through hydrogen bonding, dipole-dipole etc. The removal of acetyl forms a thermoreversible elastic gel (Du et al. 2012, Herranz et al. 2013). The gel formation facilitates modified glucomannan in application as excipient of drug (Zhang et al. 2014). Extensive studies have been conducted in gelation mechanism of deacetylated glucomannan (DGM). This DGM has been attempted to be applied in sausage & seafood product (Solo-de-Zaldivar et al. 2014), wound dressing (Huang et al. 2015) and DNA excipient (Wen et al. 2008).

Porang tuber (Amorphophallus oncophyllus) is known as an excellent local source of glucomannan which grown commonly on the border of Indonesia forest. This tuber is underutilized for direct human consumption due to the calcium oxalate content (Harmayani et al. 2014). Modification of glucomannan of A. oncophyllus and its properties was still limitedly explored. Wardhani et al. (2017) has reported significant effect of variables on the deacetylation. This study aimed to study simultaneous effect of temperature and time deacetylation on the degree of deacetylation, solubility and swelling characteristics of DGM. Since DGM has potential as a drug excipient, hence the characteristics of DGM were determined in solution which represents the pH of digestive system.

MATERIAL AND METHODS

Materials

Glucomannan of Amorphophallus oncophillus was obtained from Nganjuk, East Java (91%). Ethanol (96%), HCl, Na_2CO_3, and KOH were bought from Merck, Indonesia.

Deacetylation Process

Deacetylation was conducted following the method of Du et al. (2012). Glucomannan (10 g) was dissolved in 100 ml ethanol (75%) before reacted with Na_2CO_3 (0.4 M,100 ml). The suspension was magnetically stirred at 300 rpm. After finishing the reaction, the suspension was filtered to collect the solid part which then dried subsequently. The deacetylation was conducted at various temperatures (room temperature, 40, 50, and 60°C). Each of the temperatures was run at different times (2, 4, 8, 16, 24 and 28 h). The dried samples were washed three times with 50 ml ethanol (50, 70 and 96%). After drying, the samples were subject to DD, solubility and swelling determination.

Deacetylation Degree

Deacetylation degree (DD) was determined according to the method of Zhang et al. (2015). Dried sample of deacetylated glucomannan was placed in erlenmeyer with 10 ml ethanol (75%). After

stirring at 40° C for 30 min, KOH was added (0.5 M, 5ml). The excess of KOH was titrated with HCl (0.1 M) using phenolphthalein as an indicator. A blank was also prepared in parallel. The content of acetyl (ω_o) was calculated according to the equation [1].

$$\omega 0 = \frac{(V2 - V1) x NHCl x\, Macetyl}{ms} 100\% \quad (1)$$

where V_1 and V_2 are the volume of HCl for titration the sample and blank in liter, respectively, *NHCl* is the normality of HCl, *Macetyl* is the molecular weight of acetyl (43 g/mol) and *ms* is the mass of sample (g). DD was calculated following the formula [2]:

$$DD = \frac{\omega 0 - \omega}{\omega 0} x 100\% \quad (2)$$

where ω_0 and ω are the acetyl content in native glucomannan and in partially deacetylated sample, respectively.

Solubility and Swelling Determination

DGM was subject to solubility and swelling determination, following the method of Wardhani et al. (2017) for solubility and Daramola and Osanyinlusi (2006) for swelling. These determination were conducted in 2 pHs *e.g.* 1.2 and 6.8. DGM (0.1 g) was dissolved in 10 ml HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8) at 60°C for 30 min. The supernatant and the pasta were collected and the weights were recorded after centrifuging at 4000 rpm for 20 min. The supernatant and the paste were oven dried and weight afterward.

$$\%solubility = \frac{Weight\ of\ dried\ supernatant}{Weight\ of\ supernatan} x\ 100\% \quad (3)$$

$$swelling = \frac{weight\ of\ paste}{weight\ of\ dried\ paste} \quad (4)$$

RESULT AND DISCUSSION

In this work, glucomannan was deacetylated using Na_2CO_3 at various temperatures (room temperature, 40, 50, and 60°C). Each of the deacetylation temperatures was conducted at different times (2, 4, 8, 16, 24, and 28 h). The result was subject to DD, solubility and swelling index. These analysis were determined at pH 1.2 and 6.8. These pHs were selected to represent the pH of digestive system in the stomach and intestine (Beasley et al. 2015).

The Deacetylation Degree (DD)

The effect of temperature and time of deacetylation on DD of glucomannan is presented at **Figure 1**. Both variables showed a favorable on increasing DD. Higher temperature provides more energy to release acetyl (Wardhani et al. 2016). It also gives an energy for molecules to have a faster movement which subsequently results in more collision among them. This condition facilitates penetration into internal macromolecules and accelerate attacking other molecules (Li et al. 2014).

Deacetylation glucomannan as a function of time consists of two stages. The rapid decrease observed in the first hour when glucomannan cleavages into products followed by a slower rate degradation (Jin et al. 2014). Prolonging deacetylation period allows the higher possibility of reactants to collide each other. A significant improvement of DD was observed only at early room temperature reaction. Other than that

variable, the DD increased gradually along the deacetylation time. Li et al. (2014) suggested that deacetylation was a nucleophilic substitution reaction which not only depends on the reactant concentration but also concentration the product. After the reaction conducted for certain time, the number of acetyl groups available for reaction dropped. This condition led to slow down the reaction.

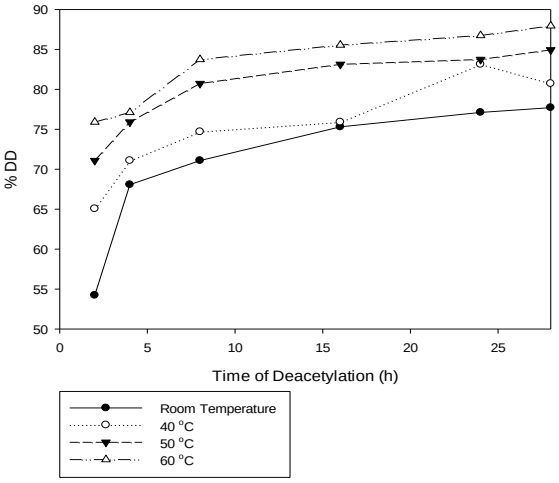

Fig. 1: The effect of deacetylation time and temperature on the deacetylation degree of glucomannan

It was hard to replace the whole acetyl group and gain 100% DD. Extending the period of deacetylation over 16h did not influence the DD significantly. The highest DD (87.95%) was obtained after deacetylation at 60°C for 28h, which was still far from the total acetyl removal. Li et al. (2014) found 92% DD after 72h deacetylation. They reported all the acetyl was still hardly removed unless the excess of alkali was added in which molar ratio between glucomannan and Na_2CO_3 is 57:1. Similar effect of deacetylation time on DD was reported by Cho et al. (2000). These deacetylations resulted the DD in the range of 54.22-87.95% which was quite similar with the result of Li et al. (2014) who found 83% DD for deacetylation glucomannan using Na_2CO_3 at 50°C for 24 h.

Glucomannan Solubility

Deacetylation of glucomannan by removing the acetyl groups using Na_2CO_3 resulted in modification of its physicochemical properties. The effect of deacetylation time and temperature on DGM solubility was determined in 2 pHs i.e. 1.2 and 6.8 (**Figure 2**). Figure 2 shows

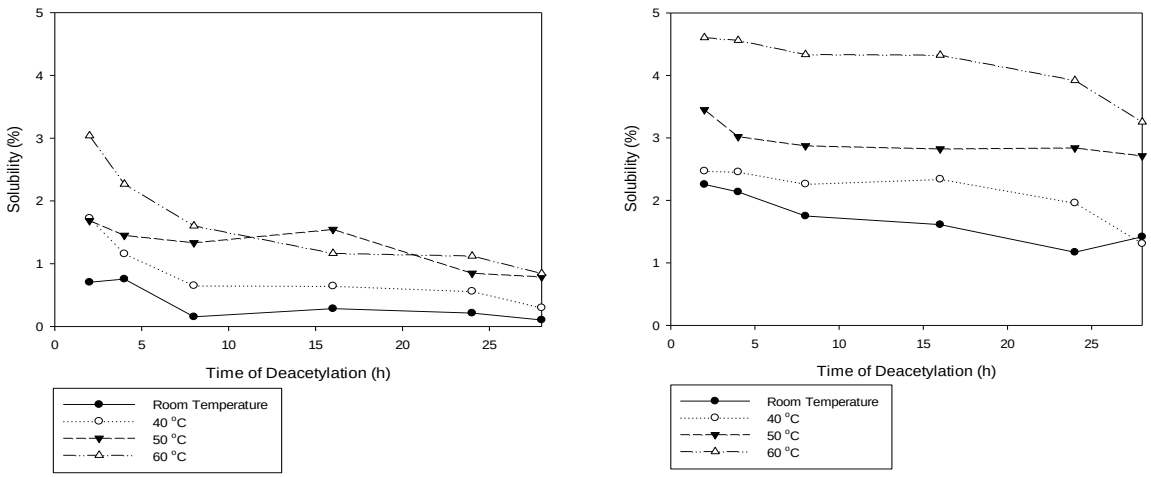

Fig. 2: The effect of deacetylation time and temperature on solubility of DGM at pH 1.2 (left) and pH 6.8 (right)

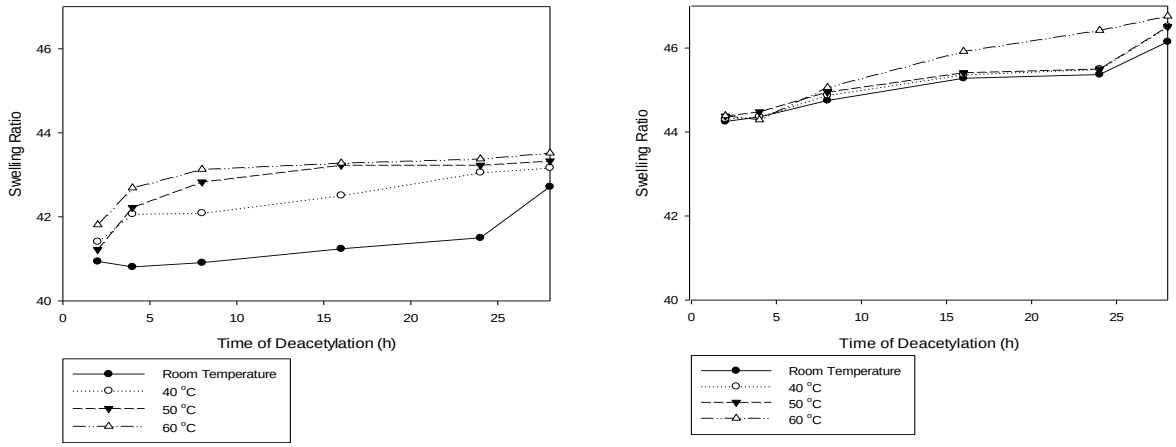

Fig. 3: The effect of deacetylation time and temperature on swelling index of DGM at pH 1.2 (left) and pH 6.8 (right).

deacetylation reduced the solubility of glucomannan. Extending deacetylation led to reducing the solubility of DGM gradually. Interestingly, DGM of higher deacetylation temperature has higher solubility. Solubility of DGM in pH 1.2 was lower than pH 6.8. The range of solubility of DGM in pH 1.2 and 6.8 was 0.103-3.03 % and 1.42-4.61%, respectively.

Various effects of DD on solubility have been reported. Chen et al. (2011) and Du et al. (2012) found solubility of DGM was insignificantly change up to 52% of DD. However, the solubility decreased significantly over 52% of DD. Similar phenomenon was reported by Li et al. (2014) which found dropped of solubility on higher DD than 60%. Base on our experiment results, we considered that the solubility of DGM partly due to effect of acetyl group removal (Wardhani et al. 2017). However, deeper study should be conducted to deliberate other factors involved in the solubility of DGM.

Glucomannan Swelling

Basically, swelling property of a material refers to the case when water molecules enter the material molecules and bind with the hydrophilic groups in the material molecules. Hence, swelling depends on the hydrophilic group of the molecules and intermolecular force (Wenling et al. 2005). The effect of deacetylation time and temperature on the glucomannan swelling at 2 (two) different pHs (1.2 and 6.8) is presented in **Figure 3**. These two pH are represented the free-acidity condition in the digestive tract particularly in stomach and intestine (Beasley et al. 2015).

In general, Figure 3 describes increasing deacetylation temperature allow DGM to have higher swelling index. Extending deacetylation time leads to improve swelling index. The swelling process of DGM consists of 2 stages, i.e., the breakage of inter-molecular hydrogen bonds of DGM followed by developing the hydrogen bonds between DGM and the water molecules. After deacetylation, less acetyl residues were available in the DGM. This indicated that DGM has more opportunities to form inter-molecular hydrogen bonds. Higher DD exhibits higher stability of DGM

due to stronger hydrogen bonds formed with water (Pan et al. 2011).

Deacetylation reduced steric hindrance and lead to increase DGM interaction (Huang et al. 2015). However, over interaction could facilitate reducing the number of site for water sorption. Chen et al. (2011) observed slightly swelling of DGM when DD over 80%. Jin et al. (2015) reported moisture absorption of DGM film increases with DD due to greater exposure of OH group which enhance binding to more water through hydrogen bonding, but extending DD over 52% reduce the absorption. Moreover, water absorption also affected by the primary structure, long-range structure and condensed states of the polymers (Jin et al. 2015).

DGM has higher swelling in pH 6.8 (44.25-46.76%) than in pH 1.2 (40.94-43.52%). Wen et al. (2009) stated that the higher the pH of swelling analysis, the higher the swelling value. It is due to the higher molecular interaction occurs in the higher pH. The higher swelling value makes the glucomannan form gel well. Hydrogen bonding is considered as the main contributor in gel formation, which influences the interaction of hydroxyl groups of sugar residues in DGM.The hydrophobic interactions, on the other hand, result from the repulsive force exerted by the aqueous environment (Wen et al. 2009). Difference swelling sensitivity of DGM support for its application as excipient of controlled-release drug.

CONCLUSION

DD was in line with deacetylation time and temperature. Solubility of the DGM decreased along with the deacetylation time. A reverse trend was found for swelling determination at both pHs. Interestingly, the swelling and solubility were lower at pH 1.2 than those at pH 6.8. These results showed functional properties of DGM was pH-sensitive. This property showed a potency of DGM as an excipient of controlled-release drug delivery system.

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