

**THESIS**

**DEPOLIMERISASI GLUKOMANAN PORANG  
(*Amorphophallus muelleri* Blume) DENGAN SELULASE**

**DEPOLYMERIZATION OF PORANG GLUCOMANNAN  
(*Amorphophallus muelleri* Blume) WITH CELLULASE**



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**AGRICULTURAL PRODUCT TECHNOLOGY STUDY  
PROGRAM  
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FACULTY OF AGRICULTURE  
SRIWIJAYA UNIVERSITY  
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This thesis was written to fulfill one of the requirements to accomplish S1 degree of Agricultural Technology at Faculty of Agriculture, Sriwijaya University



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## SUMMARY

**HUBERTUS JUDEA ENGGARDY.** Depolymerization of Porang Glucomannan (*Amorphophallus Muelleri* Blume) with Cellulase (Supervised by **ANNY YANURIATI** and **FRISKA SYAIFUL**).

Glucomannan with lower molecular weight (MW) has the ability of prebiotics and high functional food. Reducing MW of glucomannan can be done by enzymatic depolymerization. Enzymatic depolymerization of glucomannan proved to be more effective using cellulase.

This research aimed to depolymerize glucomannan enzymatically with cellulase to produce oligosaccharides, and to study the effect of enzyme concentrations and durations of depolymerization on intrinsic viscosity, molecular weight, degree of polymerization (DP), water solubility index, water holding capacity (WHC), and oil holding capacity (OHC) of depolymerized glucomannan (DGM). This study used a Factorial Completely Randomized Design with two factors. The first factor was the concentration of cellulase (5 and 10 mg/ml) and the second factor was the durations of depolymerization (30,60,90, and 120 minutes). The observed parameters were intrinsic viscosity, molecular weight, degree of polymerization (DP), water solubility index, water holding capacity (WHC), and oil holding capacity (OHC).

The results showed that cellulase concentration significantly affected the decrease in intrinsic viscosity, MW, DP, and WHC, but also significantly affected an increase in the water solubility index and OHC of DGM. Durations of depolymerization significantly affected the decrease in intrinsic viscosity, MW, DP, WHC and OHC, but also significantly affected an increase in the water solubility index of DGM. The interaction of enzyme concentration and durations of depolymerization had a significant effect on the decrease in intrinsic viscosity, MW, DP and OHC, but also significantly affected an increase in the water solubility index of DGM. The interaction of enzyme concentration and durations of depolymerization that had not produced oligosaccharides was probably because the enzyme concentration was too high and the durations for depolymerization were too long.

Keywords: depolymerization, cellulase, porang glucomannan

## **APPROVAL SHEET**

### **DEPOLYMERIZATION OF PORANG GLUCOMANNAN (*Amorphophallus muelleri* Blume) WITH CELLULASE**

. As one of the requirements to accomplish S1 degree of Agricultural Technology at Faculty of Agriculture, Sriwijaya University

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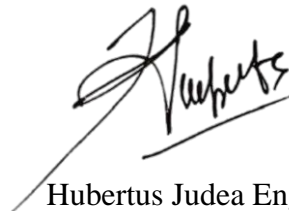
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Indralaya, July 2021



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## **BIOGRAPHY**

The writer was born in Palembang, South Sumatera province on October 11<sup>th</sup>, 1999. The writer is the third child of three children of Mr. Ir. Edy Rahwono and Mrs. Yosephine Ega Widya Prabawati.

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The writer expects that this thesis can contribute ideas that are useful for the readers and in the development of science. The writer realize that this thesis still has many shortcomings, therefore suggestions and comments from the readers are needed so that this thesis can be even better.

Indralaya, July 2021

Writer

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# CHAPTER 1

## INTRODUCTION

### 1.1. The Background

Carbohydrate compounds based on the number of constituents can be grouped into three, namely Monosaccharides, Oligosaccharides, and Polysaccharides. Monosaccharides are carbohydrate compounds consisting of one molecule with five or six carbon atoms and they are also called monomers. Oligosaccharides are carbohydrate compounds composed of 2-10 monosaccharide molecules, while polysaccharides are composed of more than 10 monosaccharides. The important role of polysaccharides in food is as a texture enhancer and as a source of energy. Polysaccharides, like other polymeric compounds, can also be hydrolyzed with the help of specific enzymes (Winarno, 2008).

One of the polysaccharides that play a role in the characteristics of foodstuffs, such as improving texture and viscosity is glucomannan. Glucomannan is a polysaccharide composed of D-glucose and D-mannose. The bonds between monomers in glucomannan compounds are  $\beta$ -1, 4- glycosidic and some branches are connected by  $\beta$ -1, 6- glycosidic bonds. Besides, the average molecular weight of glucomannan is  $10^4$  Da to  $2 \times 10^6$  Da (Jiang *et al.*, 2018).

Glucomannan with low molecular weight has high prebiotic ability and some processed foods that are high in fiber need to use glucomannans with low molecular weights so that their functional food capabilities increase. Depolymerized glucomannan (DGM) has advantages as a natural prebiotic and antioxidant (Jiang *et al.*, 2018).

Polysaccharide depolymerization can be done by several methods, one of which is enzymatic. Enzymatic depolymerization is done by using enzymes that work specifically on the hydrolyzed polysaccharide. Enzymatic hydrolysis of glucomannan can be done with the help of cellulose enzymes. The cellulose enzyme ( $\beta$ -glucanase) hydrolyzes the glucomannan at  $\beta$ -1,4-glycosidic bonds (Jiang *et al.*, 2018).

The advantages of the enzymatic method compared to other methods are producing higher extracts, reproducibility, environmentally friendly and efficient in the energy use. The concentration of enzymes in the depolymerization process has an effect on producing optimal of DGM. Liu *et al.* (2015) found that DGM with the lowest intrinsic viscosity was obtained from treatment concentration of the enzyme mananase as much as 150 U/g, while the enzyme cellulose produced optimal DGM at a concentration of 15 mg/ml (Al-Ghazewi *et al.*, 2007). The depolymerization time that produced DGM with a polymerization degree of 10-70 was 3 hours (Al-Ghazewi *et al.*, 2007). This study focused on the concentration of cellulose enzymes and the right time to depolymerize glucomannan from porang tubers (*A. muelleri* Blume). The glucomannan used was from porang tubers which were a potential food crop as a source of glucomannan in Indonesia.

### **1.2. The Objectives**

The aims of this study were 1) to depolymerize glucomannan enzymatically with cellulose to produce oligosaccharides and 2) to find out the effect of cellulose enzyme concentration and depolymerization time on intrinsic viscosity, molecular weight, degree of polymerization, water solubility index, water holding capacity, and oil holding capacity of DGM.

### **1.3. Hypothesis**

Enzymatic depolymerization of glucomannan with cellulose enzymes could produce oligosaccharides. Besides, the concentration of cellulose enzymes and depolymerization time significantly affected the intrinsic viscosity, molecular weight, degree of polymerization, water solubility index, water holding capacity and oil holding capacity of DGM.

## CHAPTER 2 LITERATURE REVIEW

### 2.1. Porang (*Amorphophallus muelleri* Blume)

Porang tubers are the most widely used part of the porang plant (*Amorphophallus muelleri* Blume). Porang has a tuber that is classified as single because only one tuber is produced in every single porang tree. Porang tuber diameter can reach 28 cm with a weight of 3 kg at the age of 1 year. The inside of the tuber is brownish yellow and the outside is dark brown (Saleh *et al.*, 2015). The shape and color of porang tubers could be seen in Figure 1.



Source: Perwitasari, 2020

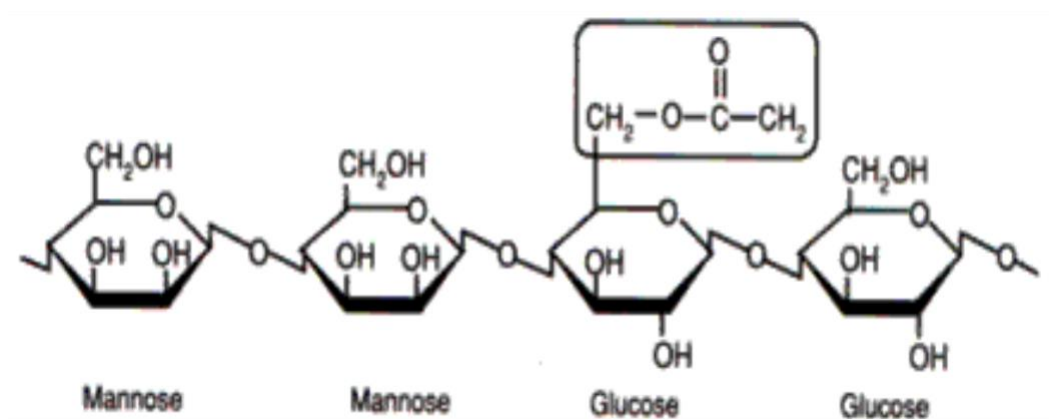
Figure 1. Porang Tubers (*A. muelleri* Blume)

Porang tubers have a polysaccharide component in the form of glucomannan. The content of glucomannan in porang tubers was quite high, which was 15-64% on a dry basis (Nugraheni *et al.*, 2018). The optimum age of tubers for glucomannan extraction was 2 years. Besides glucomannan, porang tubers contain calcium oxalate. Calcium oxalate content in 1 year old porang tubers with an average weight of 100 g could reach 0,19% (Wahyuni *et al.*, 2020). Calcium oxalate in porang tubers could cause itching and if consumed in excess, it would inhibit the absorption of calcium in the body. The highest formation of calcium oxalate occurred in the mid-growth phase (Ardhian dan Indriyani, 2013).



## 2.2. Glucomannan

The structure of glucomannan consists of the monomers D-glucose dan D-mannose. Glucomannan also has an acetyl group every 10 carbon group units at positions C2, C3, and C6 (Katsuraya *et al.*, 2003). The acetyl group in glucomannan plays a role in the solubility of glucomannan. Glucomannan is naturally abundant in porang tubers (Saleh *et al.*, 2015). The chemical structure of glucomannan could be seen in Figure 2.



Source: Nugraheni *et al.*, 2018

Figure 2. Chemical Structure of Glucomannan

The use of glucomannan in the food sector is as an additive that is useful for thickening food. Yanuriati *et al.* (2017) state that the ability of glucomannan, namely to expand, form a gel, thicken, absorb and hold water, can improve the physical properties of food products, especially texture and rheology. Besides, Tester and Al-Ghazzewi (2013) assert that the ability of glucomannan as an additive in food also has a positive impact on health, such as lowering cholesterol, triglycerides, and blood glucose. The prebiotic properties of glucomannan are beneficial for the large intestine or colon tissue as an energy source, stimulate the growth of lactic acid bacteria (LAB), and reduce the growth of pathogens.

## 2.3. Depolymerization

Depolymerization can be defined as an action to change the chemical structure of a compound in the form of a polymer to be simpler, such as

polysaccharides into oligosaccharides or monosaccharides. The main purpose of depolymerization is to obtain a lower molecular weight of a polymer compound (Sandria *et al.*, 2017).

There are 3 methods of depolymerization, namely chemical depolymerization, physical depolymerization, and enzymatic depolymerization. Chemical depolymerization utilizes chemical compounds to break bonds in the polymer to be depolymerized, such as ozone compounds (O<sub>3</sub>), sodium nitrite (NaNO<sub>2</sub>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Physical depolymerization is done by using ultrasonic waves, while enzyme depolymerization is done with the help of enzymes that are specific to the substrate (Rokhati *et al.*, 2015).

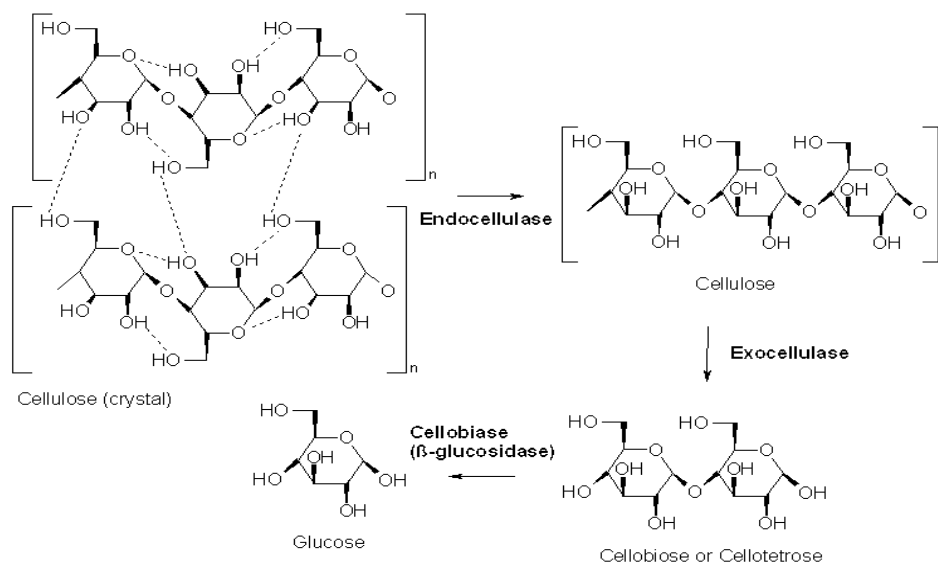
Chemical and physical depolymerization has weakness compared to enzyme depolymerization. The weakness of the chemical depolymerization method is that it produces residues that can harm the environment because it uses high concentrations of chemical compounds. The chemical depolymerization method has a low yield and the molecular weight is difficult to control. The physical depolymerization has a weakness that it requires special device such as an autoclave (Rokhati *et al.*, 2015). The advantages of using the enzymatic depolymerization method are that it is environmentally friendly because it does not use high concentrations of chemical compounds, produces high yields, works specifically for high specificity results, and it is light in operation because the reaction is easy to control (Rokhati *et al.*, 2015).

Al-Ghazewi *et al.* (2007) found that glucomannan depolymerization could be done with cellulose enzyme (C013P, 3000 U/g, Biocatalysts, Pontypridd, United Kingdom) with incubation time was 2-4 hours and enzyme concentration was 10, 15, 20, 25 mg/ml. Optimal hydrolyzate result was obtained by incubating glucomannan flour in acetate buffer (200 mmol/L pH 4.5) with a ratio of 1:10. The depolymerization time that produced DGM with polymerization degree (10-70) was 3 hours at 60 °C, while the concentration of the cellulose enzyme that produced the optimal hydrolyzate was 15 mg/ml.

## 2.4. Cellulase Enzyme

Glucomannan depolymerization can be done with two enzymes. They are cellulase and mannanase because the molecular structure allows for double cutting. The difference between cellulase and mannanase enzymes is in their hydrolysis mechanism. Mannanase enzymes work randomly to catalyze the hydrolysis of  $\beta$ -1,4-manosidic bonds, while cellulase enzymes can break  $\beta$ -1,4-glycosidic bonds to release glucose in glucomannan (Jiang *et al.*, 2018). Commercial cellulase enzyme products commonly used in the hydrolysis of glucomannan are a mixture of endoglucanase, exoglucanase, and glucosidase.

Al-Ghazzewi and Tester (2012) state that endoglucanase can break the  $\beta$ -1,4-glycosidic bonds between glucose. Exoglucanase can break 1,4-glucopyranose bonds in the non-reducing site and glucosidases can break down cellobiose into glucose monomers (Jiang *et al.*, 2018). Glucomannan which is hydrolyzed by the cellulase enzyme has an advantage over mannanase, which is that it can be completely fermented by probiotics in the human colon. Cellulase had been shown to be more effective at hydrolyzing glucomannan than mannanase (Al-Ghazzewi & Tester, 2012). The mechanism of the cellulase enzyme could be seen in Figure 3.



Source: Sutikno *et al.*, 2016

Figure 3. Mechanism of Hydrolysis by Cellulase Enzymes

## CHAPTER 3

### METHODOLOGY

#### 3.1. Place and time

This study had been carried out at the Chemical Laboratory of Agricultural Products and Microbiology Laboratory of Agricultural Products, Faculty of Agriculture, Sriwijaya Univeristy. It had been done from March 2021 to July 2021.

#### 3.2. Tools and Materials

The tools used in this study were: 1) aluminum foil, 2) 80 mesh sieve, 3) ball pipette, 4) Philip blender (HR115), 5) aluminum cup, 6) desiccator, 7) measuring cup, 8) hot plate, 9) cuvette, 10) Erlenmeyer flask, 11) Max Blend magnetic stirrer (6 multi point), 12) Analytical balance of Fujitsu (FS AR210) 13) Memmert oven (UN 55 53L), 14) dropper pipette, 15) measuring pipette, 16) Oregon centrifuge (LC 04C Plus), 17) falcon tube, 18) digital timer, 19)Ubbelohde viscometer, 20) vortex, and 21) Memmert waterbath shaker type GFL 1083.

The materials used in this study were: 1) deionized water, 2) buffer sodium acetate 50 mM pH 5,0, 3) powdered cellulase enzyme from *Aspergillus niger* 1180U/g optimum pH 5,0 and optimum temperature 37°C brand Sigma-Aldrich 1U equivalent to the amount of enzyme that liberates 1 $\mu$ mol glucose, 4) corn oil, and 5) porang glucomannan flour.

#### 3.3. Research method

The experimental design in this study used a factorial Completely Randomized Design (CRD) method with two treatment factors, namely (A) cellulase enzyme concentration consisting of 2 treatment levels and (B) depolymerization time consisting of 4 treatment levels. Each treatment was repeated 3 times. Each treatment referred to Connolly *et al.* (2010) as follows:

1. Cellulase Enzyme Concentration (A):

A1 = 5 mg/ml

A2 = 10 mg/ml

## 2. Depolymerization Time (B):

B1 = 30 minute

B2 = 60 minute

B3 = 90 minute

B4 = 120 minute

**3.4. Statistic analysis**

The data in this study were processed using analysis of variance (ANSIRA) quantitatively with parametric statistical analysis techniques. Based on Hanafiah (2002), the general model of factorial RAL with 2 treatment factors was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

keterangan:

 $Y_{ijk}$  = observation value $\mu$  = average value $\alpha_i$  = the effect of cellulase enzyme concentration $\beta_j$  = effect of depolymerization time $(\alpha\beta)_{ij}$  = the interaction effect of cellulase enzyme concentration and depolymerization time $\varepsilon_{ijk}$  = error

Analysis of variance in statistics was shown in Table 3.1

**Table 3.1. ANSIRA List of Factorial Completely Randomized Design (CRD)**

Source of Diversity	Free Degrees	Number of Squares	Number of Squares	F-count	F-Table
(SD)	(FD)	(NS)	Middle		5%
Treatment (T)	$V_1 = (m.n) - 1$	JKP	$JKP/V_1$	KTP/KTG	$(V_1, V_2)$
Factor A	$V_2 = m - 1$	JKA	$JKA/V_2$	KTA/KTG	$(V_2, V_5)$
Factor B	$V_3 = n - 1$	JKB	$JKB/V_3$	KTB/KTG	$(V_3, V_5)$
Interaction of AB	$V_4 = (m-1)(n-1)$	JKAB	$JKAB/V_4$	KTAB/KTG	$(V_4, V_5)$
Error	$V_5 = V_6 - V_1$	JKG	$JKG/V_5$		
Total	$V_6 = (m.n.r) - 1$	JKT	$JKT/V_6$		

Source: Hanafiah, 2002

The determination of the significance of each treatment in this study was done by comparing the F-table at the level of 5% on the ANSIRA results with the F-count based on the following comparison:

1. If  $F\text{-table } 5\% \geq F\text{-count}$ , it means that there was no significant effect (<sup>ns</sup>)
2. If  $F\text{-table } 5\% < F\text{-count}$ , it means that there was a significant effect (\*)

Further tests were carried out if the F-count in ANSIRA was greater than the F-table at the level of 5 % by using the Duncan's multiple range test (DMRT). A further DMRT test was carried out to determine the average difference for each experiment. The general formula for DMRT according to Hanadiah (2002) was:

$$\text{DMRT } \alpha = P_{d, 0,05(p,v)} \times S_y$$

$$S_y = \sqrt{\frac{\text{CSQ}}{K}}$$

Note :

$P_{\alpha(p,v)}$  = Standard P- value at test level  $\alpha$  and error free degree  $v$

$S_y$  = common mean standard error

CSQ = Center Square Error

$r$  = Number of treatments

The diversity coefficient (DC) test was done to test the level of accuracy in this study. Hanafiah (2002) stated that to have good accuracy if the DC was less than 15%. The value formula for the diversity coefficient was:

$$\text{DC (\%)} = \sqrt{\frac{\text{CSQ}}{Y}} \times 100\%$$

Notes:

DC = Diversity Coefficient

CSQ = Center Square Error

Y = the average value of all experimental data

### **3.5. Procedure**

The procedures of this study consisted of enzyme preparation and glucomannan depolymerization, as follows:

#### **3.5.1. Enzyme Preparation**

The preparation of powdered cellulase enzyme was carried out according to the way Nieves et al. (1998) with modifications to the amount of enzyme, as follows:

1. The powdered cellulase enzyme was weighed as much as 0.50847 g (Appendix 8).
2. The enzyme was dissolved in 50 mM sodium acetate buffer with pH 5.0 to a weight of 3 g at 37°C.
3. Steps 1-3 were repeated with the powdered cellulase enzyme weighing 1.01694 g (Appendix 8).

#### **3.5.2. Glucomannan Depolymerization**

The enzymatic depolymerization of glucomannan was carried out according to the way Al-Ghazewi et al. (2007) with modifications, as follows:

1. 4 g of porang glucomannan flour was dissolved in 50 mM sodium acetate buffer with pH 5.0 to 37 g (10% w/w) in a closed Erlenmeyer flask.
2. The glucomannan solution and the enzyme solution were put into a water bath shaker until they reached the optimum temperature for the Sigma-Aldrich cellulase enzyme, which was  $37\pm 1^\circ\text{C}$ .
3. The cellulase enzyme was mixed into the glucomannan solution with the appropriate concentration of treatment (A).
4. The sample was incubated in a water bath shaker for the incubation time according to treatment (B). The temperature of the water bath shaker WAS maintained at the optimum temperature, which was  $37\pm 1^\circ\text{C}$  and a speed of 50 revolutions/minute.
5. After incubation, the enzyme was inactivated by inserting an Erlenmeyer flask containing a glucomannan solution into boiling water ( $100^\circ\text{C}$ ) for 10 minutes.
6. The sample was put into a 50 ml falcon tube, then dried with a freeze dryer.
7. DGM solids were ground in a blender and then sieved through an 80 mesh sieve.

### 3.6. Parameter

Parameters observed in this study include physical characteristics, namely intrinsic viscosity, molecular weight, degree of polymerization, water solubility index, water holding capacity, and oil holding capacity.

#### 3.6.1. Intrinsic Viscosity

The intrinsic viscosity of DGM was measured based on the working method from Tatirat *et al.* (2012) and Kishida *et al.* (2014) with the modifications to the concentration of the mother liquor, as follows:

1. The sample was dissolved in 1% deionized water and the dissolution was carried out for 24 hours at room temperature.
2. The sample solution was centrifuged for 60 minutes at a speed of 2500 rpm.
3. The supernatant portion was taken and made 5 series of dilutions.
4. The supernatant viscosity was measured with 5 concentrations using the Ubbelohde Viscometer at 30°C.
5. Relative viscosity was determined by the following equation:

$$\text{Relative Viscosity} = \frac{t}{t_0}$$

Notes:

t = sample flow time

t<sub>0</sub> = solvent flow time

6. The reducing viscosity was determined by the following equation:

$$\text{Reducing viscosity} = \frac{(\eta_{\text{rel}} - 1)}{C}$$

Notes:

η<sub>rel</sub> = relative viscosity

C = sample concentration

7. The graph of the reduced viscosity (y) against the sample concentration (x) was made using Microsoft Excel application.
8. The linear equation obtained was  $y = ax + b$ , where a was the slope and b was the intercept. The intrinsic viscosity value was expressed through the intercept value of the linear equation of the reduced viscosity graph (y) against the sample concentration (x).



### 3.6.2. Molecular Weight

The determination of the molecular weight of DGM was calculated based on the procedure from Kishida et al. (2014) with the Mark-Houwink-Sakurada equation, as follows:

$$[\eta] = K_M \square MW^a$$

Notes:

$\eta$  = intrinsic viscosity

$K_M$  = Mark-Houwink-Sakurada Coefficient ( $6,37 \times 10^{-4}$ )

$MW$  = molecular weight

$a$  = constant (0,74)

### 3.6.3. Degree of Polymerization (DP)

DP of DGM was calculated based on the formula described in Shrivastava (2018), as follows:

$$DP = \frac{M_w}{M_o}$$

Notes:

DP = Polymerization Degree

$M_w$  = Polymer molecular weight DGM

$M_o$  = Glucose monomer molecular weight

### 3.6.4. Water Solubility Index

The water solubility index of DGM was measured based on the work of Du *et al.* (2012), as follows:

1. 0.1 g of the sample was weighed and dissolved in 24.9 ml of deionized water.
2. The solution was stirred for 1 hour with a magnetic stirrer.
3. Samples were centrifuged for 20 minutes at 4000 rpm.
4. 10 g of supernatant was taken and dried at 105°C until the weight was constant.
5. The water solubility index was calculated based on the following equation of Du *et al.* (2012).

$$\text{Water Solubility Index} = \frac{m \times FP}{W} \times 100\%$$

Notes:

m = weight of dissolved components in 10 g supernatant

FP = Dividing Factor ( $\frac{\text{Mother solution weight (g)}}{\text{Supernatant Weight(g)}}$ )

W= total weight of glucomannan

### 3.6.5. Water Holding Capacity

Water holding capacity (WHC) of DGM was measured based on the working method from Koroskenyi and McCarthy (2001) with the following modifications:

1. 0.1 g of dry DGM sample was dissolved in 14 ml of deionized water in a known weight falcon tube.
- 2.. The DGM solution was homogenized with a vortex for 2 minutes and then the tube was covered with aluminum foil and incubated for 1 hour.
3. Samples were centrifuged for 60 minutes at a speed of 2500 rpm.
4. The clear supernatant was discarded and the residue (subnatant) in the falcon tube was weighed.
5. WHC was calculated by the formula, as follows:

$$\text{WHC} = \frac{(\text{Residual weight} - \text{empty tube}) - (\text{Sample weight})}{\text{Sample weight}}$$

### 3..6.6. Oil Holding Capacity

The oil holding capacity (OHC) of DGM was measured based on the AACC (2000) method with the following modifications:

1. 15 ml falcon tube was weighed and the weight was recorded.
2. Corn oil was put into a falcon tube as much as 3.5 ml and 0.5 g of DGM samples was added.
3. The solution was homogenized with a vortex for 2 minutes.
4. After being homogeneous, the sample was centrifuged for 60 minutes at a speed of 2500 rpm.
5. The supernatant was removed slowly and the residue (subnatant) in the falcon tube was weighed.
6. OHC was calculated by the following equation:

$$\text{OHC} = \frac{(\text{Residue weight} - \text{empty tube}) - (\text{Sample weight})}{\text{Sample weight}}$$

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1. Intrinsic Viscosity

According to Kaneshima et al. (2012) one of the characteristics of polymer molecules was intrinsic viscosity which was defined as an increase in the viscosity of a solution with the addition of solute per unit mass at infinite dilution. The intrinsic viscosity of glucomannan before depolymerization was 31.4 ml/g and decreased to 0.27 ml/g after the addition of cellulase enzyme 5 mg/ml for 30 minutes (Appendix 1. Table 1).

Analysis of diversity was only carried out for glucomannan samples treated with enzymes. The results of the analysis of diversity showed that the enzyme concentration, depolymerization time, and the interaction of the two treatments had a significant effect on the intrinsic viscosity of DGM (Appendix 2. Table 2.3). Based on the results of Duncan's further test the effect of enzyme concentration on intrinsic viscosity (Table 4.1), the addition of enzyme concentration per 5 mg/ml could significantly reduce the intrinsic viscosity of glucomannan.

Table 4.1 Further test of Duncan's Multiple Range Test of Enzyme Concentration Effect on Intrinsic Viscosity of DGM

Enzyme Concentration	Average Intrinsic Viscosity	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
10 mg/ml	0,02 ml/g		a
5 mg/ml	0,13 ml/g	0,0006229	b

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

According to Liu *et al.* (2015), the intrinsic viscosity of glucomannan would decrease as the concentration of enzymes reacted in the depolymerization process increased. Cellulase enzymes in the glucomannan depolymerization process could break the  $\beta$ -1,4-glycosidic bonds so that they converted polysaccharides into oligosaccharides or monosaccharides (Jiang *et al.*, 2018). The more cellulase enzymes that were reacted would increase contact with polysaccharides so as to produce more monosaccharides (Wardhani *et al.*, 2021).

The results of Duncan's further test of the depolymerization time effect on intrinsic viscosity could be seen in Table 4.2. The addition of depolymerization time every 30 minutes could significantly reduce the intrinsic viscosity of glucomannan. During 120 minutes of depolymerization, a significant decrease in intrinsic viscosity occurred greater at the longer time of depolymerization.

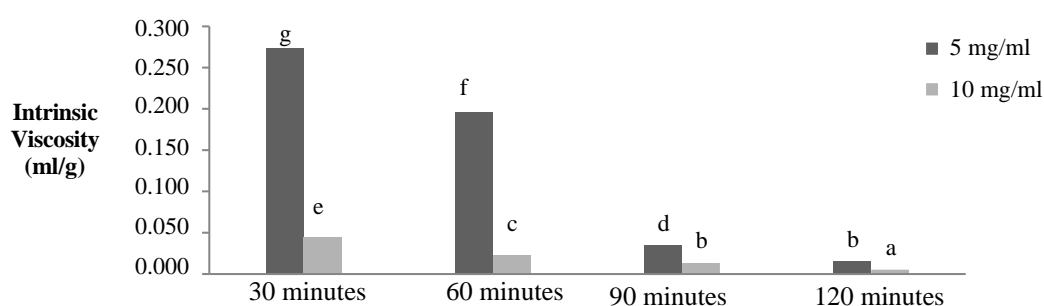
Table 4.2. Further test of Duncan's Multiple Range Test of Depolymerization Time Effect on Intrinsic Viscosity of DGM

Depolymerization Time	Average Intrinsic Viscosity	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
120 minutes	0,01 ml/g		a
90 minutes	0,02 ml/g	0,001246	b
60 minutes	0,11 ml/g	0,001308	c
30 minutes	0,16 ml/g	0,001300	d

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

Based on Liu *et al.* (2015), depolymerization by the mannanase enzyme resulted in a decrease in the viscosity of glucomannan. Based on research by Al-Ghazewi *et al.* (2007) cellulase enzyme was able to produce optimal DGM at a concentration of 15 mg/ml for 3 hours.

The results of Duncan's further test of the interaction effect between cellulase enzyme concentration and depolymerization time on the intrinsic viscosity of DGM was shown in Figure 4.1. Glucomannan depolymerized with cellulase enzyme as much as 5 mg/ml for 30 minutes had the highest intrinsic viscosity. During 120 minutes of depolymerization, the decrease in the intrinsic viscosity of glucomannan was significantly greater at a higher enzyme concentration and a longer depolymerization time.



### Depolymerization Incubation Time

Figure 4.1. Histogram of the interaction effect of cellulase enzyme concentration and depolymerization time on the intrinsic viscosity of DGM. The treatments followed by the same letter showed no significant difference ( $p > 0.05$ ).

Wardhani *et al.* (2021) asserted that glucomannan was composed of glucose and mannose monomers linked by  $\beta$ -1.4-glycosidic bonds. Cellulase enzyme from *Aspergillus niger* (0.3 U/mg) of Sigma Aldrich successfully degraded glucomannan molecules for 300 minutes resulting in low intrinsic viscosity. The intrinsic viscosity of the glucomannan solution (1% w/v) was decreased by 2.865 ml/g using 20 ppm cellulase.

#### 4.2. Molecular Weight

Enzymatic depolymerization was proved that it could reduce the molecular weight of glucomannan. Yin *et al.* (2020) found that a significant decrease in the molecular weight of glucomannan occurred in the first 10 minutes of the depolymerization process using the endo-1.4- $\beta$ -mannanase enzyme. The molecular weight of glucomannan before depolymerization was 2195897,42 Da and decreased to 3598,15 Da after the addition of cellulase enzyme (5 mg/ml) for 30 minutes (Appendix 1. Table 1).

The results of the diversity analysis showed that the enzyme concentration, depolymerization time, and the interaction of the two treatments had a significant effect on the molecular weight of DGM (Appendix 3. Table 3.3). Based on the results of Duncan's further test of the enzyme concentration effect on the molecular weight of DGM (Table 4.3), the addition of the enzyme concentration of 5 mg/ml could significantly reduce the molecular weight of glucomannan.

Table 4.3. The Further Test of Duncan's Multiple Range Test of Enzyme Concentration Effect on Molecular Weight of DGM

Enzyme Concentration	Average Molecular Weight	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
10 mg/ml	126,40 Da		a
5 mg/ml	1543,57 Da	6,4952229	b

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

Glucomannan had a high molecular weight because it was composed of D-Glucose and D-Mannose monomers to form a long polymer. The molecular weight of glucomannan ranged from  $10^4$  Da to  $2 \times 10^6$  Da. Cellulase enzymes hydrolyzed

$\beta$ -1.4-glycosidic bonds in glucomannans to produce oligosaccharides or monosaccharides. Glucomannan that had been hydrolyzed into oligosaccharides or monosaccharides had a lower molecular weight (Al- Ghazewi *et al.*, 2007; Al- Ghazzewi and Tester, 2012).

The results of Duncan's further test of the depolymerization time effect on the molecular weight of DGM could be seen in Table 4.4. The addition of depolymerization time every 30 minutes could significantly reduce the value of the glucomannan molecular weight. For 120 minutes of depolymerization, a significant decrease in molecular weight occurred greater at the longer depolymerization timer.

Table 4.4. Further test of Duncan's Multiple Range Test of Depolymerization Time Effect on Molecular Weight of DGM

Depolymerization Time	Average Molecular Weight	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
120 minutes	44,12 Da		a
90 minutes	140,19 Da	12,99045	b
60 minutes	1203,61 Da	13,63997	c
30 minutes	1952,06 Da	13,98638	d

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

Chen *et al.* (2016) found that the average molecular weight of enzymatically depolymerized glucomannan with several time variations experienced a significant decrease. Besides, Glucomannan experienced a significant decrease in molecular weight with longer depolymerization time. The longer depolymerization time caused the hydrolysis of  $\beta$ -1.4- glycosidic bonds in the glucomannan polymer to be more intensive. The average value of the molecular weight of DGM could be seen in Figure 4.2.

The results of Duncan's further test the effect of the interaction between cellulase enzyme concentration and depolymerization time on the molecular weight of DGM is shown in Figure 4.2. Glucomannan depolymerized with cellulase enzyme as much as 5 mg/ml for 30 minutes had the highest molecular weight. During 120 minutes of depolymerization, the decrease in glucomannan's BM was significantly greater at a higher enzyme concentration and a longer depolymerization time.

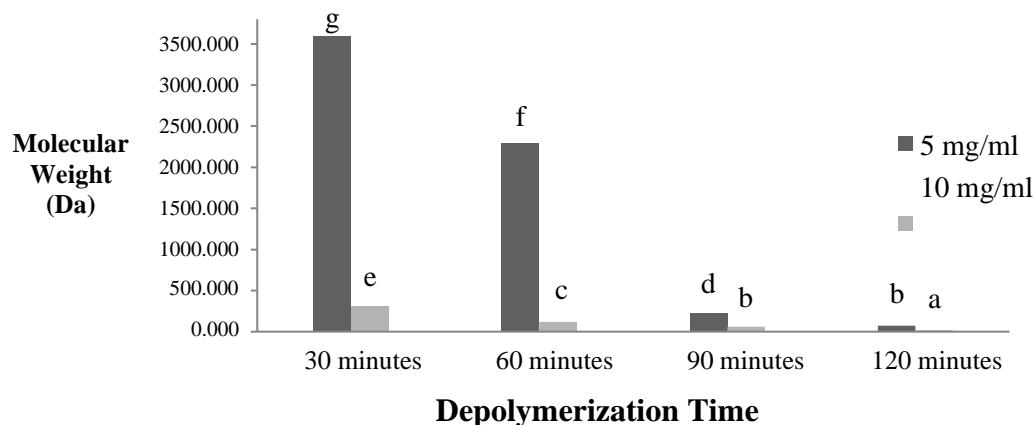


Figure 4.2. Histogram of the interaction effect of cellulase enzyme concentration and depolymerization time on the molecular weight of DGM. Treatment followed by the same letter showed no significantly different ( $p > 0.05$ ).

The results of this study were in line with the research of Al-Ghazewi *et al.* (2007) who found that the molecular weight of glucomannan hydrolyzate decreased with increasing enzymes concentration and depolymerization time. Cellulase enzymes that reacted with glucomannan polymer would break the  $\beta$ -1.4-glycosidic bond during the depolymerization process. Similarly, Safaria *et al.* (2013) stated that the cellulase enzyme added to the substrate caused an interaction that would form an enzyme-substrate complex. The enzyme-substrate complex formed would produce new products in the form of oligosaccharides or monosaccharides. A longer interaction between the enzyme and the substrate would cause the reaction to run more maximally up to 8 hours.

### 4.3. Polymerization Degree

Habibah *et al.* (2013) stated that the degree of polymerization was the number of repeating units in a polymer chain. The longer the polymer chain, the higher the degree of polymerization. The degree of glucomannan polymerization before depolymerization was 12187.24 and decreased to 19.97 after the addition of 5 mg/ml cellulase enzyme for 30 minutes (Appendix 1. Table 1).

The results of diversity analysis showed that the enzyme concentration, depolymerization time and the interaction of the two treatments significantly affected the degree of polymerization of DGM (Appendix 4. Table 4.3). Based on Duncan's further rest results on the effect of enzyme concentration (Table 4.5), the addition of enzyme concentration per 5 mg/ml could significantly reduce the degree of glucomannan polymerization.

Table 4.5. Further Test of Duncan's Multiple Range Test of Enzyme Concentration Effect on the Polymerization Degree of DGM

Enzyme Concentration	Average Polymerization Degree	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
10 mg/ml	0,70		a
5 mg/ml	8,57	0,03605	b

Note : The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p>0.05$ ).

Anggela *et al.* (2020) found that porang glucomannan was successfully depolymerized enzymatically into shorter chain molecules. Cellulase enzymes that broke the  $\beta$ -1,4-glycosidic bonds in glucomannan (Al-Ghazewi and Tester, 2012) changed the polymer chains into shorter ones. Glucomannan depolymerized with a higher enzyme concentration has a lower degree of polymerization, presumably because more  $\beta$ -1,4-glycosidic bonds could be broken by the cellulase enzyme.

Therefore, the results of Duncan's further test of the depolymerization time effect on the degree of polymerization of DGM could be seen in Table 4.6. The addition of depolymerization time every 30 minutes could significantly reduce the value of the glucomannan polymerization degree. For 20 minutes of depolymerization, the decrease in the degree of polymerization was significantly greater at a longer depolymerization time.

Table 4.6. Further test of Duncan's Multiple Range Test of Depolymerization Time on Polymerization Degree of DGM

Depolymerization Time	Average Polymerization Degree	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
120 minutes	0,24		a
90 minutes	0,78	0,0721	b
60 minutes	6,68	0,0757	c
30 minutes	10,83	0,0776	d

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p>0.05$ ).

The DGM polymerization degree was calculated based on its molecular weight. The degree of polymerization of DGM was directly proportional to the molecular weight of DGM. DGM which had a low molecular weight would have a lower degree of polymerization than DGM with a high molecular weight. Cellulase enzymes interacted with glucomannan to form an enzyme-substrate complex.



According to Safaria *et al.* (2013), a longer incubation time for 0 to 8 hours caused the interaction of the cellulase enzyme with a polymer to be maximized so as to produce products that had shorter chains. The shorter the chain of a polymer was, the lower the degree of polymerization was.

Duncan's further test results showed that the interaction effect of enzyme concentration and depolymerization time on the degree of polymerization of DGM (Figure 4.3), glucomannan depolymerized with cellulase enzyme as much as 5 mg/ml for 30 minutes had the highest degree of polymerization. For 120 minutes of depolymerization, the decrease in the degree of glucomannan polymerization was significantly greater at a higher enzyme concentration and a longer depolymerization time.

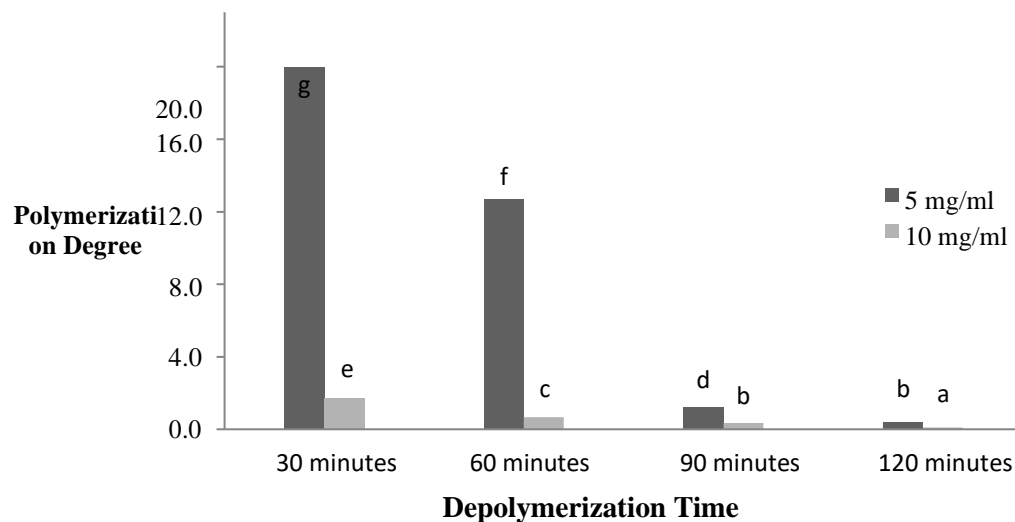


Figure 4.3. Histogram of the interaction effect of cellulase enzyme concentration and depolymerization time on the degree of polymerization of DGM. The treatments followed by the same letter showed no significant difference ( $p > 0.05$ ).

The degree of oligosaccharides polymerization ranged from 2-10 (Winarno, 2008), while the degree of polymerization of DGM resulting from treatment interactions did not reach that range. Oligosaccharides were not formed presumably because the high concentration of cellulase enzymes and too long depolymerization time could break the  $\beta$ -1,4-glycosidic bonds in glucomannan more maximally (Safaria *et al.*, 2013). The use of cellulase enzyme concentration and lower depolymerization time was probably able to depolymerize glucomannan into oligosaccharides.

#### 4.4. Water Solubility Index

Natural glucomannan had a high molecular weight, which was  $1 \times 10^6$  Da, so it took a longer time to dissolve in water. Water solubility index was influenced by the molecular weight and morphology of glucomannan (Yanuriati *et al.*, 2017). Glucomannan which had a low molecular weight had a higher particle porosity so that it was more soluble in water (Luo *et al.*, 2012). The presence of an acetyl group in glucomannan also affected the water solubility index of glucomannan (Davé and McCarthy, 1997). The water solubility index of glucomannan before depolymerization was 40.97% and increased to 67.22% after the addition of cellulase enzyme (5 mg/ml) for 30 minutes (Appendix 5. Table 5.1).

Moreover, the results of diversity analysis showed that the enzyme concentration, depolymerization time and the interaction of the two treatments significantly affected the water solubility index of DGM (Appendix 5. Table 5.3). Furthermore, the results of Duncan's further test of the enzyme concentration effect on the water solubility index (Table 4.7) showed that the addition of the enzyme concentration (5 mg/ml) could significantly increase the water solubility index of glucomannan.

Table 4.7. Further Test of Duncan's Multiple Range Test of the Enzyme Concentration Effect on Water Solubility Index of DGM

Enzyme Concentration	Average Solubility Index	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
5 mg/ml	68,86 %		a
10 mg/ml	77,84 %	0.16	b

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

Glucomannan which had a low molecular weight had a high water solubility index (Luo *et al.*, 2012). The molecular weight of glucomannan depolymerized with 10 mg/ml cellulase enzyme was lower than glucomannan depolymerized with 5 mg/ml cellulase enzyme. Therefore, the water solubility index of the depolymerized glucomannan at the cellulase enzyme concentration of 10 mg/ml was higher than the cellulase enzyme concentration of 5 mg/ml.

Molecular weight affected the water solubility index of glucomannan. The results of this study were in line with Luo *et al.* (2012) that glucomannan with lower molecular weight had a higher water solubility index than glucomannan with high molecular weight.

Besides, glucomannan with low molecular weight had more porous surface morphology than glucomannan with high molecular weight. The more porous glucomannan particles had weak hydrogen bonds so that they were easily soluble in water (Luo *et al.*, 2012; Yanuriati and Basir, 2020).

Therefore, the results of Duncan's further test of the depolymerization time effect on the water solubility index could be seen in Table 4.87. The addition of depolymerization time every 30 minutes could significantly increase the water solubility index of glucomannan. For 120 minutes of depolymerization, the increase in water solubility index was significantly greater at a longer depolymerization time.

Table 4.8. Further Test of Duncan's Multiple Range Test of Depolymerization Time Effect on Water Solubility Index of DGM

Depolymerization Time	Average Solubility Index	SSD <sub>0.05</sub>	DMRT <sub>0.05</sub>
30 minutes	71,16 %		a
60 minutes	72,23 %	0,3188	b
90 minutes	73,04 %	0,3347	c
120 minutes	76,99 %	0,3432	d

Note: The treatments followed by the same letter in the DMRT<sub>0.05</sub> column showed no significant difference ( $p > 0.05$ ).

The water solubility index was inversely proportional to the molecular weight of DGM. The molecular weight of DGM depolymerized for 30 minutes was significantly higher than that of DGM depolymerized for 60 minutes. DGM which was depolymerized for 120 minutes had the lowest molecular weight so that it had the highest water solubility index (Table 4.8).

The results of Duncan's further test of the interaction effects between cellulase enzyme concentration and depolymerization time on the water solubility index of DGM was shown in Figure 4.4. The addition of cellulase enzyme (5 mg/ml) for 30 minutes caused an increase in the value of the water solubility index of glucomannan from 40,97% to 67,22% (Appendix 5. Table 5.1). The water solubility index of glucomannan increased significantly with each increase in concentration of 5 mg/ml and the addition of depolymerization time for 30 minutes. For 120 minutes of depolymerization, the water solubility index tends to be higher at the longer depolymerization time at the same enzyme concentration.

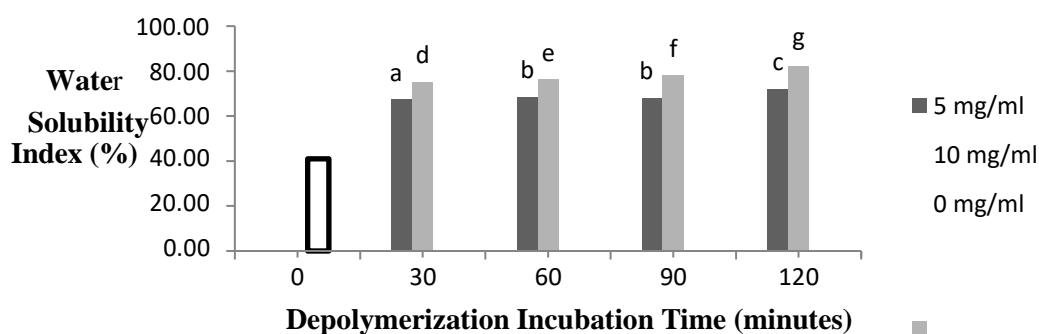


Figure 4.4. Histogram of the interaction effect of cellulase enzyme concentration and depolymerization time on the water solubility index of DGM. The treatments followed by the same letter showed no significant difference ( $p > 0.05$ ). Controls were not analyzed for variance

The solubility index of DGM was inversely proportional to its molecular weight. The molecular weight of DGM decreased with the addition of enzyme concentration and depolymerization time (Al-Ghazewi *et al.*, 2007). The water solubility index of low molecular weight DGM was higher than that of high molecular weight DGM (Luo *et al.*, 2012).

#### 4.5. Water Holding Capacity

The value of water holding capacity (WHC) was related to the water solubility index of glucomannan. Glucomannan with a high water solubility index had weak hydrogen bonds. Weak hydrogen bonds between the hydroxyl groups in reducing sugars and H atoms in water molecules caused low water absorption, so the WHC value would decrease. (Kohyama *et al.*, 1996; Yanuriati *et al.*, 2017). WHC glucomannan before depolymerization was 33.75 g water/g glucomannan and decreased to 15.84 g water/g glucomannan after addition of cellulase enzyme (5 mg/ml) for 30 minutes (Appendix 6. Table 6.1)

The results of the analysis of diversity showed that the enzyme concentration and depolymerization time had a significant effect on WHC of DGM, while the interaction of the two treatments had no significant effect on WHC of DGM. (Appendix 6. Table 6.3). Based on Duncan's further test results on the effect of enzyme concentration (Table 4.9), the addition of the enzyme concentration of 5 mg/ml could significantly reduce glucomannan WHC.

Table 4.9. Further Test of Duncan's Multiple Range Test of Enzyme Concentration Effect on WHC of DGM

Enzyme Concentration	Average of WHC	SSD <sub>0,05</sub>	DMRT <sub>0,05</sub>
10 mg/ml	8,68 g air/g DGM		a
5 mg/ml	11,21 g air/g DGM	0.19	b

Note: The treatments followed by the same letter in the DMRT<sub>0,05</sub> column showed no significant difference ( $p > 0.05$ ).

The WHC value of glucomannan was inversely proportional to the value of the water solubility index of glucomannan. Glucomannan depolymerized with cellulase enzyme concentration of 10 mg/ml had a higher water solubility index so that it had a lower WHC value than glucomannan depolymerized with cellulase enzyme concentration of 5 mg/ml. The decrease in WHC value in glucomannan which had a higher water solubility index was caused by weak hydrogen bonds between the hydroxyl group on the glucomannan reducing sugar and the H atom in the water molecule. (Kohyama *et al.*, 1996).

Moreover, the results of Duncan's further test of depolymerization time effect on WHC could be seen in Table 4.10. The addition of depolymerization time every 30 minutes could significantly reduce the WHC value of glucomannan. For 120 minutes of depolymerization, the decrease in WHC glucomannan was significantly greater at a longer depolymerization time.

Table 4.10. Further Test of Duncan's Multiple Range Test of Depolymerization Time Effect on WHC of DGM

Depolymerization Time	Average of WHC	SSD <sub>0,05</sub>	DMRT <sub>0,05</sub>
120 minutes	6,37 g air/g DGM		a
90 minutes	8,82 g air/g DGM	0,3803	b
60 minutes	10,51 g air/g DGM	0,3993	c
30 minutes	14,07 g air/g DGM	0,4095	d

Note: The treatments followed by the same letter in the DMRT<sub>0,05</sub> column showed no significant difference ( $p > 0.05$ ).

Furthermore, WHC was inversely proportional to the water solubility index of DGM. DGM depolymerized for 120 minutes had the highest water solubility index so that it had the lowest WHC.

The hydrogen bonds in glucomannans with a low water solubility index were weaker than the hydrogen bonds in glucomannans with a higher water solubility index. The weaker the hydrogen bond between the hydroxyl group and water in glucomannan caused the WHC value to decrease (Yanuriati *et al.*, 2017).

#### 4.6. Oil Holding Capacity

Oil holding capacity (OHC) of a food ingredient was one of the important characteristics to improve the taste of the food. The OHC value was influenced by the surface properties and particle size of a material (Fleury and Lahaye, 1991; Odoemelam, 2003). Grigelmo-Miguel and Martín-Belloso (1998) asserted that food fiber that had an OHC value of 0.86 – 1.27 g oil/g sample was suitable to be added to foodstuffs with a high percentage of fat and emulsion. The OHC of glucomannan before depolymerization was 1.29 g oil/ g glucomannan and increased to 2.44 g oil/ g glucomannan after the addition of cellulase enzyme (5 mg/ml) for 30 minutes (Appendix 7. Table 7.1)

The results of diversity analysis showed that the enzyme concentration, depolymerization time, and the interaction of the two treatments had a significant effect on OHC of DGM (Appendix 7. Table 7.3). Based on the results of Duncan's further test the effect of enzyme concentration on OHC of DGM (Table 4.11), the addition of the enzyme concentration of 5 mg/ml could significantly increase the OHC of glucomannan.

Table 4.11. Further Test of Duncan's Multiple Range Test of Enzyme Concentration Effect on OHC of DGM

Enzyme Concentration	Average of OHC	SSD <sub>0,05</sub>	DMRT <sub>0,05</sub>
5 mg/ml	1,77 g minyak/g DGM		a
10 mg/ml	2,06 g minyak/g DGM	0,0011	b

Note: The treatments followed by the same letter in the DMRT<sub>0,05</sub> column showed no significant difference ( $p > 0.05$ ).

The porous surface structure of glucomannan could also affect the value of oil holding capacity. The pores in glucomannan could absorb oil. The more pores on the surface of glucomannan, the more oil that could be absorbed (Herlina *et al.*, 2016). Luo *et al.* (2012) stated that the pores in glucomannan with low molecular weight were more numerous than in glucomannan with high molecular weight, thereby it increased the holding capacity of glucomannan oil.

Cellulase enzyme with a concentration of 10 mg/ml produced DGM with a lower molecular weight than a concentration of 5 mg/ml, so it had a higher oil holding capacity.

Besides, the results of Duncan's further test of the depolymerization time effect on OHC of DGM could be seen in Table 4.12. The addition of depolymerization time every 30 minutes could significantly reduce the OHC value of glucomannan. For 120 minutes of depolymerization, the decrease in OHC of glucomannan was significantly greater at a longer depolymerization time.

Table 4.12. Further Test of Duncan's Multiple Range Test of Depolymerization Time Effect on OHC of DGM

Depolymerization Time	Average of OHC	SSD <sub>0,05</sub>	DMRT <sub>0,05</sub>
120 minutes	1,30 g oil/g DGM		a
90 minutes	1,77 g oil/g DGM	0,0022	b
60 minutes	2,06 g oil/g DGM	0,0023	c
30 minutes	2,54 g oil/g DGM	0,0023	d

Note: The treatments followed by the same letter in the DMRT<sub>0,05</sub> column showed no significant difference ( $p > 0.05$ ).

The results of this study were not in line with Herlina et al. (2016) where the OHC value of gembili glucomannan (*Dioscorea esculenta* L.) would increase with the addition of depolymerization time. The pores in glucomannan could also increase the oil-binding capacity (Herlina *et al.*, 2016). The water solubility index was inversely proportional to the OHC of DGM value. OHC of DGM with high water solubility index was lower than OHC of DGM with low water solubility index. The addition of depolymerization time every 30 minutes could increase the water solubility index so that the OHC value decreases.

The OHC value in glucomannan could also be influenced by the presence of non-polar molecules. Non-polar molecules could bind large amounts of oil (Thanatcha and Pranee, 2011). The acetyl group in the chemical structure of glucomannan was non-polar. According to Putri et al. (2016), the acetyl group was non-polar because it prevented the formation of hydrogen bonds between the hydroxyl group in the reducing sugar and the H atom in the water molecule. The decrease in OHC value with increasing depolymerization time was thought to be due to the reduction of non-polar molecules in the resulting DGM.

The results of Duncan's further test of the interaction effect between cellulase enzyme concentration and depolymerization time on OHC of DGM was shown in Figure 4.5. OHC glucomannan increased with the addition of the enzyme concentration of 5 mg/ml during the first 30 minute, but decreased significantly with each additional 30 minutes of depolymerization time at the same enzyme concentration. For 120 minutes of depolymerization, the OHC of glucomannan tended to be lower at the longer depolymerization time at the same enzyme concentration.

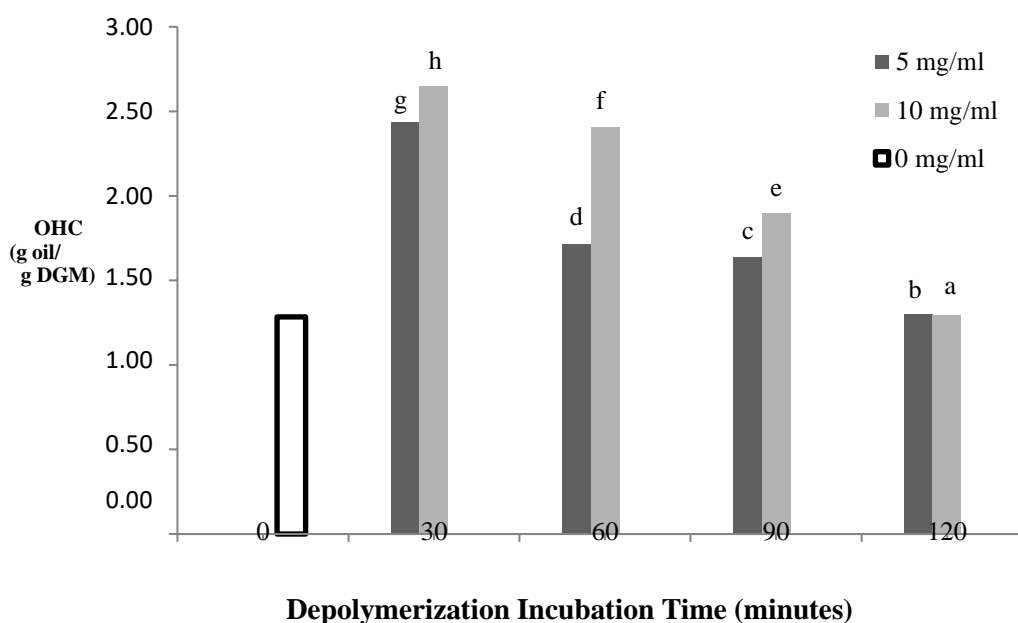


Figure 4.5. Histogram of the interaction effect of cellulase enzyme concentration and depolymerization time on OHC of DGM. The treatments followed by the same letter showed no significant difference ( $p > 0.05$ ). Controls were not analyzed for variance.

According to Herlina *et al.* (2016), the pores in the polysaccharide affected the oil holding capacity of the polysaccharide. Lower molecular weight of DGM had higher porosity (Luo *et al.*, 2012), so that the highest OHC value was found in glucomannan which was depolymerized with cellulase enzyme concentration of 10 mg/ml for 30 minutes.



## **CHAPTER 5**

### **CONCLUSIONS AND SUGGESTION**

#### **5.1. Conclusions**

The conclusions of this study were:

1. Cellulase concentration significantly affected the decrease in intrinsic viscosity, molecular weight, degree of polymerization, and WHC, but also significantly increased the water solubility index and OHC of DGM.
2. Depolymerization time significantly affected the decrease in intrinsic viscosity, molecular weight, degree of polymerization, WHC and OHC, but also significantly increased the water solubility index of DGM.
3. The interaction of enzyme concentration treatment and depolymerization time significantly affected the decrease in intrinsic viscosity, molecular weight, degree of polymerization and OHC, but also significantly increased the water solubility index of DGM.
4. The interaction of cellulase enzyme concentration treatment and depolymerization time that had been carried out had not produced oligosaccharides, presumably because the enzyme concentration was too high and the depolymerization time was too long.

#### **5.2. Suggestion**

Based on the results of this study, to produce oligosaccharides it is suggested to use glucomannan depolymerization treatment with a lower concentration of cellulase enzyme and depolymerization time.

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