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Research Article
Carboxymethylation of Glucomannan from Porang Tuber (*Amorphophallus oncophyllus*) and the Physicochemical Properties of the Product

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Abstract

Objective: This study was conducted to describe the synthesis of carboxymethyl porang glucomannan (CPGM) and its physicochemical characteristics. **Materials and Methods:** The CPGM was synthesized by treating porang glucomannan (PGM) with sodium chloroacetate under basic conditions at different temperatures (50, 60 and 70°C) for different durations (20, 40 and 60 min). The CPGM products were then analyzed to determine what functional groups were present, the degree of substitution (DS), the water solubility, the zeta potential and the viscosity. **Results:** Carboxymethylation of the porang glucomannan was confirmed by the increase in the intensity of the carbonyl absorption peak in the fourier transform infrared (FTIR) spectra. Compared to the native porang glucomannan, the carboxymethylated porang glucomannan was more negatively charged and more water soluble but was less viscous. Both temperature and reaction time influenced the viscosity and water solubility, but the DS value was more influenced by reaction time than by temperature. **Conclusion:** Carboxymethylation of porang glucomannan yielded a more useful product than did native porang glucomannan. The increase in water solubility and decrease in viscosity made the product widely applicable in the food industry. The negatively charged polymer has the potential to interact with positively charged polymers to develop new products. Further research may be conducted to tune the reaction time to achieve certain properties that will suit additional needs.

Key words: Porang glucomannan, carboxymethylation, water solubility, viscosity, degree of substitution

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Glucomannan is natural polymer consisting of β-D-mannose and β-D-glucose linked by β-1,4-glycosidic bonds[1]. Commercially, the most widely used glucomannan is isolated from the konjac tuber (*Amorphophallus konjac*), a plant that is found in Asia. Its high gelation content and viscosity are the key properties of konjac glucomannan that make it widely applicable in the food industry. It is used to thicken syrups, jellies, edible films and noodles and is used as a sausage binder. Recently, glucomannan from the porang tuber (*Amorphophallus oncophyllus)* has been developed and cultivated in Indonesia, but there have been relatively few studies on its properties and potential applications possibly because it is less soluble than commercially available konjac glucomannan. Therefore, chemically modifying the porang tuber glucomannan may be the best way to enhance its properties.

Among the various modification methods that aim to replace a hydroxyl group with a carboxymethyl group, carboxymethylation stands out. Several studies have shown that carboxymethylation of konjac glucomannan increased its solubility, lowered its viscosity and made the polymer anionic[2-4]. These improved properties make carboxymethyl glucomannan a good starting material for developing film formations, encapsulating enzymes and strengthening paper, as well as acting as a drug carrier and metal ion adsorber[2,5-8].

Generally, the level of modification from carboxymethylation can be assessed by the degree of substitution (DS). There are several factors that influence DS: concentration of the base and etherification agent (monochloroacetic acid), reaction temperature, reaction period, solvent, water content and polymer type[9-12]. Optimizing temperature and reaction time may be the simplest way to tune the DS. In konjac glucomannan, the roles of those two parameters have been studied. The role of temperature, reaction time and sodium acetate concentration in modifying the DS of carboxymethyl konjac glucomannan has been studied by Xiao *et al.*[13], but their study did not show the impact of each treatment on the DS value. They also used a long reaction time (1-3 h) to get a DS between 0.2 and 0.4. Wang *et al.*[2] reported that high DS values (0.45-0.7) could be reached using the same temperature (65°C) for several short reaction periods (25, 40, 55 and 70 min). In this study, various temperatures and short reaction times were evaluated to get different DS values in the carboxymethylation of porang glucomannan. The impact of different DS values on solubility, viscosity and zeta potential was also studied.

MATERIALS AND METHODS

Materials: The PGM with a viscosity of 40,000-80,000 mPa s was prepared by the research team from the Faculty of Agricultural Technology, Universitas Gadjah Mada. Sodium monochloroacetate and sodium hydroxide were acquired from Sigma-Aldrich (St. Louise, USA), while hydrochloric acid, nitric acid and acetic acid were purchased from JT Baker Chemical Co. (Phillipsburg, New Jersey).

Preparation of CPGM: CPGM was prepared following the procedure described by Wang *et al.*[2] with different reaction temperatures, T (50, 60 and 70°C) and reaction times, t (20, 40 and 60 min), to get CPGM with different degrees of substitution. Samples were denoted as CPGM (T,t). For example, CPGM synthesized using a reaction temperature of 50°C for 20 min was denoted as CPGM 5020.

FTIR spectroscopic analysis: The FTIR was performed to confirm the formation of CPGM. The FTIR spectra of CPGM and PGM were recorded on a Shimadzu 8201 PC spectrophotometer in the region between 4,000 and 400 cm^{-1}.

Degree of substitution (DS): The degree of substitution was determined by the standard method of potentiometric back-titration[14].

Water solubility: Solubility was analyzed using a procedure described by Wang *et al.*[15] with some modifications. Briefly, 1 g of CPGM was added to 100 mL of distilled water and stirred for 1 h. The dispersed solution was then centrifuged at 3,500 rpm for 20 min. The residue was dried at 105°C until a constant weight was reached. The solubility of CPGM (So) was calculated using the equation:

$$So\ (\%) = \frac{W_0 - W_r}{W_0} \times 100$$

where, W_0 is the mass of CPGM added to the distilled water and W_r is the mass of the residue after centrifugation.

Zeta potential: Zeta potential measurements of CPGM and chitosan were conducted using a Malvern Zetasizer Nanoseries (nano ZS ver 6.20, Malvern Instruments Ltd., Malvern, UK) based on the principle of phase analysis light scattering. Approximately 0.4 g of CPGM (at pH 7) was suspended in 100 mL of distilled water and filtered through nylon cloth. After the samples were loaded into the instrument, they were equilibrated for approximately 120 sec[16].

Viscosity: Viscosity was measured with an RV series Brookfield viscometer[17]. Approximately 1 g of CPGM was added to 100 mL of distilled water and stirred for 1 h and then the solution was left to stabilize for 1 h. Viscosity was then tested at different shear rates, namely, 0.5, 1, 2.5, 5, 10, 20 and 50 sec^{-1}, at room temperature.

Statistical analysis: For statistical studies, SPSS 16.0 software was used. Data were analyzed using a one-way analysis of variance (ANOVA). Means were compared using Duncan's multiple range test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Proof of carboxymethylation: Figure 1 shows the FTIR spectra of PGM and CPGM, which confirms that the carboxymethylation reaction was successful. The absorption peak at 1635 cm^{-1} is attributed to the C=O stretching vibration (from the carbonyl groups) and the absorption peak at 3410 cm^{-1} is due to the -OH stretching vibration. After carboxymethylation, the peak at 1635 cm^{-1} became stronger in most of the samples. However, there were new peaks at 1627 cm^{-1} for CPGM 7040 and CPGM 6020 and at 1604 cm^{-1} for CPGM 6060. These peaks indicate that carboxymethylation generates additional carbonyl groups in other positions besides where the hydroxyl group was. The added C=O stretching from the carbonyl groups influences the -OH stretching vibration. This can be seen in the increased intensity of the absorption peak at 3410 cm^{-1} and the new peaks at 3448, 3417, 3425, 3441 and 3371 cm^{-1}.

In konjac glucomannan, the characteristic absorption peaks at 1631, 3436[2] and 1600 cm^{-1} [7] of the carboxymethylation product became more intense. In this research, an increase in intensity of the absorption peak at 1026 cm^{-1} was also observed due to the stretching vibrations of alkyl ethers (C-O-C) formed during carboxymethylation, as shown in Fig. 2. The same characteristic peaks were found in carboxymethylated cellulose from sago waste[14].

Effect of temperature and reaction time on DS value: Most of the DS values were not significantly influenced ($p>0.05$) by an increase in the reaction temperature (can be seen by looking down the columns of Table 1), except when the reaction was carried out at 60°C for 20 min ($p<0.05$). This may be because the reaction temperatures were not high enough for the molecules to reach the activation energy for the reaction or because the temperatures could not trigger the diffusion of the etherification reagents. As a result, the reaction rate was not increased and DS was not affected[12].

The effect of reaction time on DS can be seen by looking across the rows of Table 1. At the same temperature (50°C), DS increased when the reaction time was extended from 20 or 40 min to 60 min. Meanwhile, DS decreased significantly when the reaction time was extended from 20-60 min at 60°C and from 20-40 min at 70°C ($p<0.05$). From these results, it can be concluded that at higher temperatures, DS began to decrease at shorter reaction times. Compared to the effect of temperature, reaction time was more influential on the DS of CPGM.

Research on carboxymethylation of konjac glucomannan found that the DS increased when the reaction was carried out at 65°C with various reaction times (25, 40, 55 and 70 min)[2]. A longer reaction time led to better mixing of the reactants and improved diffusion of the etherification agent into the PGM molecules[11,12,14]. A higher reaction temperature with the same reaction time may increase degradation of the polymer, leading to a decrease in DS values[11,14].

Table 1: Effect of temperatures and reaction times on degree of substitution, water solubility and zeta potential of carboxymethyl porang glucomannan (CPGM)

	Reaction time (min)		
Reaction temperature (°C)	20	40	60
Degree of substitution			
50	0.21 ± 0.02^{a1}	0.20 ± 0.02^{a1}	0.26 ± 0.03^{b1}
60	0.27 ± 0.02^{a2}	0.24 ± 0.02^{ab1}	0.22 ± 0.02^{b1}
70	0.29 ± 0.01^{a2}	0.21 ± 0.01^{b1}	0.23 ± 0.02^{b1}
Water solubility			
50	29.04 ± 1.79^{a1}	27.62 ± 1.59^{a1}	59.83 ± 0.65^{b1}
60	77.92 ± 0.85^{a2}	60.29 ± 2.13^{b2}	71.47 ± 0.57^{c2}
70	84.50 ± 0.31^{a3}	90.41 ± 2.25^{b3}	93.33 ± 1.59^{b3}
Zeta potential			
50	-6.31 ± 0.75^{a1}	-6.20±0.14a1	-8.06 ± 0.73^{a1}
60	-12.88 ± 0.11^{ab2}	-12.32 ± 0.25^{b2}	-13.12 ± 0.18^{a2}
70	-13.78 ± 0.32^{a2}	-13.98 ± 0.46^{a3}	-16.02 ± 0.25^{b3}

Values within the same row with the same letters are not significantly different ($p>0.05$), Values within the same column with the same number are not significantly different ($p>0.05$)

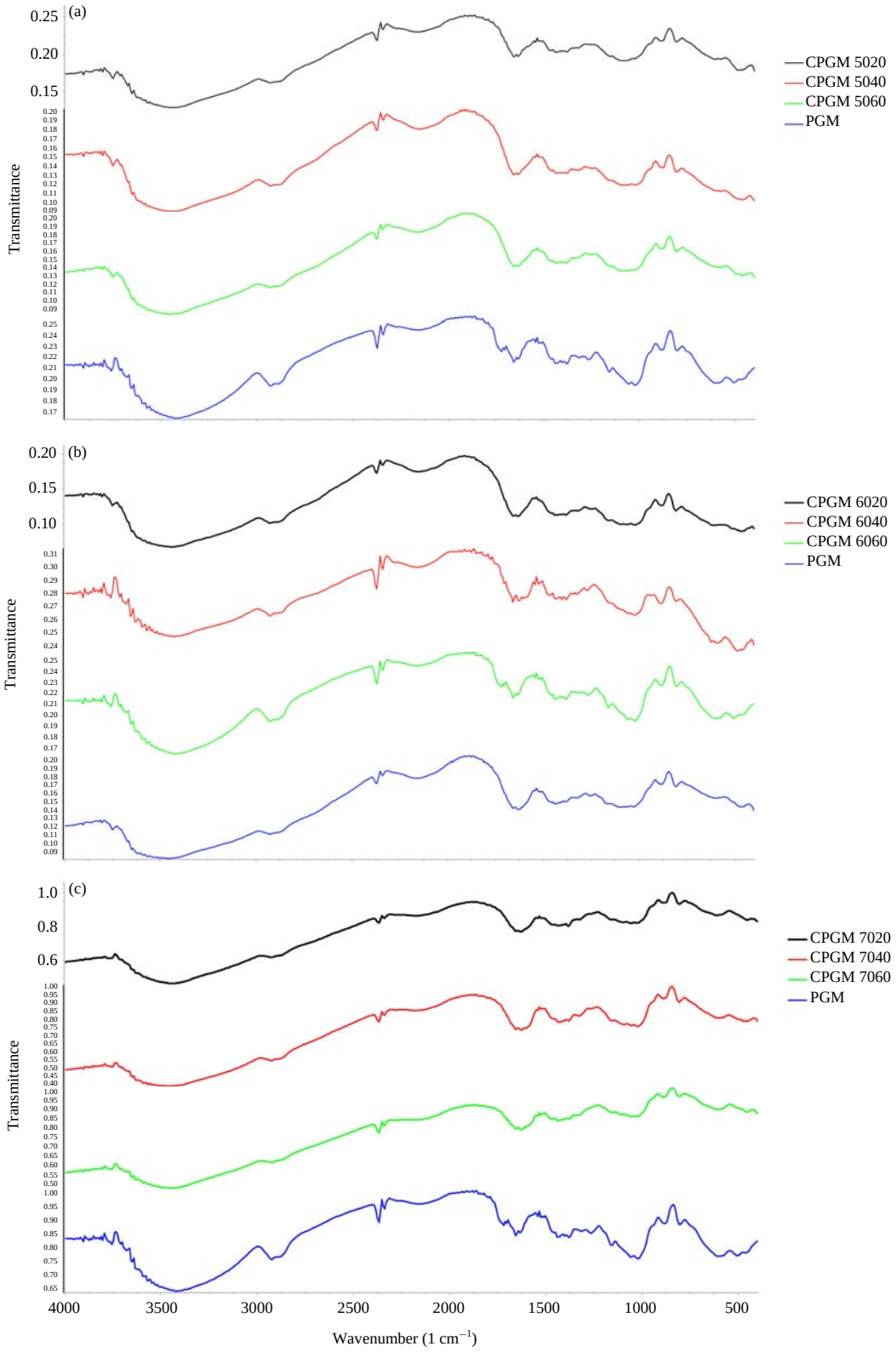

Fig. 1(a-c): FTIR spectra of carboxymethyl porang glucomannan (CPGM) and the native (porang glucomannan, PGM) produced at (a) 50°C, (b) 60°C and (c) 70°C. Each of the treatment was done for 20, 40 and 60 min (denotation of CPGM 5020 means CPGM with the treatment condition at 50°C for 20 min)

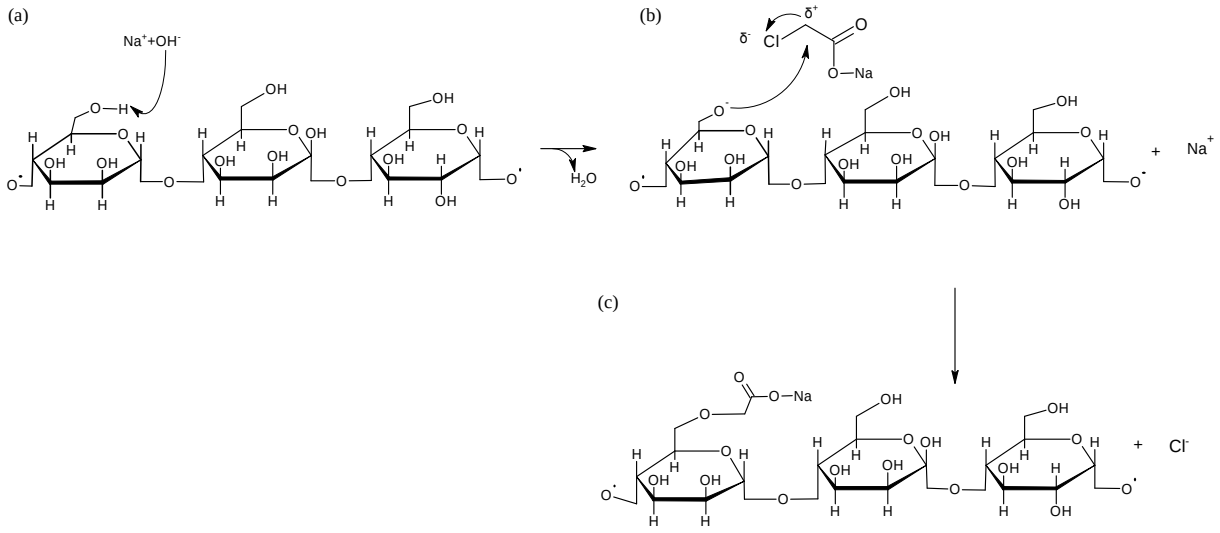

Fig. 2(a-c): Carboxymethylation reaction of glucomannan (modified from Silva *et al.*[10]), (a) Alkalization, OH^- from strong base will react with H^+ from glucomannan to form, (b) Alkoxide ion as nucleophile which will attack electrophile C from sodium monochloroacetate. At the same time, electron in the bond between C-Cl will kick off onto Cl and (c) Glucomannan has been added with carboxymethyl groups

Several studies have also reported that DS would reach its optimum value at a certain temperature or reaction time. Above those values, it would begin to decrease likely due to polymer degradation. The formation of sodium glycolate may also negatively affect this process and decrease the DS[9,11,12,14,18].

For cellulose, the degradation of the polymer was controlled by chemical elimination of water via intramolecular elimination leading to C2-C3 unsaturation or the formation of a ketone group on C2[14]. For rice starch, polymer degradation could be caused by agglomeration of the starch, which will generate a firm, sticky mass that can stop the stirrer[12].

Effect of temperature and reaction time on water solubility of CPGM: Carboxymethylation affected the characteristics of the polymer. Physically, the product was darker in colour compared to the native (PGM). Chemically, carboxymethylation also influenced the water solubility of CPGM (Table 1). In general, increases in reaction temperatures and times increased water solubility ($p<0.05$). Native porang glucomannan (purity 92.6%) had the water solubility of about 86.43%[19]. Compared to native, carboxymethyl porang glucomannan could reach higher water solubility when the reaction was carried out at 70°C for 40 or 60 min.

In this study, the increase in water solubility when the reaction time was extended is consistent with the increase in the DS values. The mechanism for improving solubility was explained by Xiao *et al.*[13]. During alkalization (the first step of the carboxymethylation process), some acetyl groups would be lost, causing the molecules to become entangled with each other and aggregate to form a multi-branch structure. This made the solubility of glucomannan lower. However, alkalization assisted in the introduction of carboxymethyl groups into the molecules and can cause the multi-branch structures to interact with other molecules, including water. That is why carboxymethylation influenced the water solubility, adding carboxymethyl groups increased the hydrophilicity of the polymer[20]. The longer reaction time used in this research provided more opportunities for additional carboxymethyl groups to be inserted into the molecule. As a result, the DS values and hydrophilicities were higher.

Increasing the temperature had a stronger influence on the water solubility than did extending the reaction time. It seems that having more molecules with energies greater than the activation energy was important for accelerating the reaction. However, the increase in water solubility with higher temperatures was not consistent with no significant changes in the DS values (except when the reaction time was 20 min). When reaction times were 40 and 60 min, water solubility increased when the reaction temperature was increased, but the DS values did not also increase. This indicates that there was an additional factor that influenced the water solubility of CPGM beyond the existence of carboxymethyl groups.

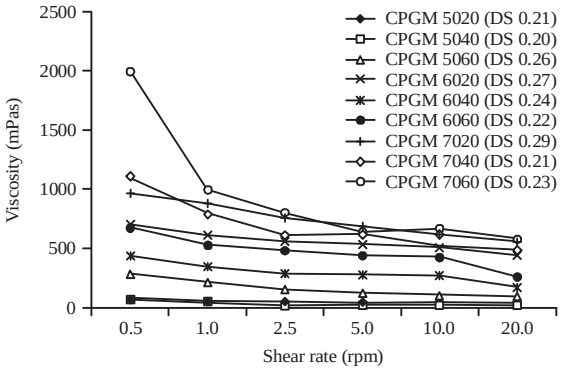

Fig. 3: Viscosities of carboxymethyl porang glucomannan in all treatments

The increase in solubility was probably influenced by changes in the interior structure of the particle from crystalline to amorphous during carboxymethylation at higher temperatures. Amorphous materials are more hygroscopic because of the ease at which they absorb water into their bulk structure in addition to surface adsorption[13].

Effect of temperature and reaction time on zeta potential of CPGM: Based on the data shown in Table 1, the zeta potentials of the CPGM products were all electronegative and they were more affected by reaction temperature than time. The negative charge was due to the insertion of a carbonyl group (COO-), which brings a negative charge[4]. The zeta potentials in this research were measured at pH 7. Du *et al.*[7] reported that the isoelectric point of glucomannan from konjac was at pH 2.69, this value is close to the pKa of a carboxylic acid, which is around 3. This means that glucomannan has negative zeta potentials above pH 2.69, which may be due to the dissociation of H^+ from the carboxylate groups in the glucomannan macromolecules. This charge caused the formation of polymer complexes through the interactions of two oppositely charged polymers[6,21-28].

Effect of temperature and reaction time on viscosity of CPGM: In general, as shear rates increased, the viscosities of all samples decreased (Fig. 3). This result proved that PGM was a pseudo-plastic non-Newtonian fluid[29]. Generally, the viscosities of the CPGM products were higher when the reaction times and temperatures were higher. This phenomenon agreed with the solubility, which followed the same trend. The increase in viscosities was due to the additional carboxymethyl groups in the molecules causing a stronger interaction between the molecules and water. These interactions clearly affected the solubility as well (as discussed earlier). Meanwhile, this interaction likely decrease the number of free water molecules, leading to increased surface tension and thus increasing the viscosity of the solution.

The viscosity of all CPGM samples was much lower than that of the native PGM. The native PGM could attain 56,000 mPa s when measured at a low shear rate (0.5 rpm) and 15,000 mPa s at a high shear rate (20 rpm). This result was similar to konjac glucomannan[3,13], cashew tree exudate polysaccharide[10] and galactomannan[9] and is likely due to polymer degradation during carboxymethylation[10]. In several studies, degradation was indicated by the presence of a larger fraction of low-molecular-weight particles[9,11]. Degradation might prevent lengthening of the polymer chain, resulting in lower molecular weights and viscosity[30]. The other possible explanation for the decrease in viscosity is that the stiffness of the molecular backbone and strong intermolecular hydrogen bonds lead to weakened hydrogen bonds between the polymers and water. This diminishes the coil expansion of the carboxymethyl group[3].

CONCLUSION

Porang glucomannan was subjected to carboxymethylation and the success of the reaction was shown by the increased intensities of the absorption peaks of the carbonyl groups in the FTIR spectra. Increasing the reaction temperature substantially increased the solubility and electronegativity of the polymer, but the DS values were more influenced by the reaction time. These conditions can be adjusted to generate polymers with desirable properties based on the needs of specific applications, making the process much more effective. To fully explore this system, more research is needed.

SIGNIFICANCE STATEMENT

This study optimized the conditions for carboxymethylation to enhance the properties of glucomannan. In this study, porang glucomannan was poorly soluble and highly viscous, therefore, its applications are limited. By subjecting porang glucomannan to carboxymethylation at high temperatures, both solubility and electronegativity can be increased. Viscosity was lower when high temperatures and reaction times were used. This study will help other researchers find new products that can enrich human lives, such as encapsulant, edible film and other product that using the interaction between more than one polymer. Additional research may be needed to effectively adjust the conditions according to the needs of each application.

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