MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Yunita *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 22 (2026) 248–259

RESEARCH ARTICLE

Effect of Degree Substitution on the Viscosity and Solubility of Carboxymethylated *Amorphophallus muelleri* Glucomannan

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Abstract Glucomannan, derived from *Amorphophallus muelleri* (AGM), is a highly viscous natural polysaccharide with reduced solubility, which has yet to be fully developed, hence constraining its application across numerous industries. Among the numerous modifications applied to AGM, carboxymethylation via the etherification process has garnered considerable interest owing to its straightforward methodology and its ability to reduce viscosity while enhancing the solubility of glucomannan. This study aims to examine the modification of *Amorphophallus muelleri* glucomannan by carboxymethylation (CMAGM) through the variation of sodium hydroxide (NaOH) and monochloroacetic acid (MCA) to ascertain the degree of substitution and its effects on viscosity and solubility. The degree of substitution was verified by titration methods, which corroborate the findings from Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) analyses. The glucomannan yield achieved through acid hydrolysis with hydrochloric acid was 52.4%. The AGM was effectively altered and validated by the emergence of a new peak at 1587–1369 cm^{-1} in the FT-IR spectra and δ = 3.3-4.1, δ = 8.057 ppm in the NMR spectra, indicative of the COOH functional group due to the modification process MCA:NaOH ratio 2:4% w/v. It was found that a lower MCA corresponds to a higher degree of substitution. Overall, the carboxymethylation effectively reduced the viscosity and enhanced the solubility of glucomannan.

Keywords: Carboxymethylated *Amorphophallus muelleri* glucomannan, sodium hydroxide, monochloroacetic acid, solubility, viscosity, degree substitution.

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Received: 21 Nov. 2025
Accepted: 23 Jan. 2026

Introduction

Glucomannan is a natural polysaccharide that consists of β-D-mannose and β-D-glucose linked by β-(1→4) glycosidic bonds. Glucomannan can be derived from the tuber of the *Amorphophallus sp,* and the Araceae family [1]. The predominant commercially available glucomannan is konjac glucomannan derived from *Amorphophallus konjac*. This species is extensively cultivated in China and Japan and is commonly consumed as food. It is also recognized by the Food Chemical Codex as a thickening, film maker, and stabilizer for the food industry [2]. Glucomannan derived from konjac possesses a greater

glucomannan content, an abundant number of hydroxyl groups (OH), and acetyl groups irregularly attached at the C-6 position of its structure. This functional group resulted in increased viscosity and solubility of the glucomannan [3]. Consequently, due to its unique physicochemical properties, *A. konjac*-derived glucomannan has found widespread application in industries such as pharmaceuticals, biotechnology, environmental engineering, and chemical sectors [4].

Recently, attention has shifted to include another species of the same genus, *Amorphophallus muelleri*, which predominantly grows in tropical regions such as Southeast Asia, including Malaysia (Figure 1). *Amorphophallus muelleri* glucomannan (AGM) has gained interest due to several properties, including gel-forming capability, swelling properties, water holding capacity, and high glucomannan content after the well-known species [5,6]. Compared to *Amorphophallus konjac*, this species offers several advantages, including tolerance to high temperatures and humidity, shorter cultivation cycles, suitability for hot climates, and strong resistance to soft rot disease [1]. However, despite its potential, relatively few studies have explored the full scope of AGM's properties and applications due to its lower solubility and higher viscosity. Consequently, chemical modification by carboxymethylation is often employed to overcome these shortcomings, especially on konjac glucomannan. It has been widely applied in various industries, such as drug development, due to its pH responsiveness [3,7], wound dressing [8], controlled release of active ingredients [2], hydrogel sensors [9], and wastewater treatment [10,11]. Nevertheless, the modification of *A. muelleri* glucomannan by carboxymethylation (CMAGM) remains relatively limited compared to other species of *Amorphophallus* [2,8,12,13].

Carboxymethylation can be a valuable strategy due to its operational simplicity, low cost, and effectiveness. The method introduces carboxymethyl groups ($–CH_2COOH$) into the glucomannan structure, reducing viscosity and imparting solubility and pH sensitivity. Previous studies have shown that carboxymethylation of glucomannan from *Amorphophallus konjac* (KGM) can be a valuable strategy due to its advantages in terms of simple operation and low-cost reagents, lower viscosity, and higher solubility [9]. This is because higher acetyl or carboxymethyl groups lead to a higher number of hydrogen bonds and increase the solubility[14]. Thus, different degrees of substitution provide different properties, specifically in the viscosity and solubility. The degree of substitution in the carboxymethylation process can be affected by various parameters, including catalyst concentration, the etherification agent (monochloroacetic acid), reaction time, and temperature. However, the degree of substitution was not significantly influenced by the temperature compared to the reaction time [15]. An earlier investigation by Aprilia *et al*. (2017) evaluated the carboxymethylation of *Amorphophallus muelleri* by altering the temperature and reaction duration to influence the degree of substitution. At 50 °C, the degree of substitution increased from 0.21 to 0.26 as the reaction time was extended from 20 to 60 min [15]. Another work by Zhu *et al*. (2023) changed the monochloroacetic acid (MCA) and reaction time for the carboxymethylation of konjac glucomannan, resulting in a higher degree of substitution compared to the previous study by Aprilia *et al* (2017). The degree of substitution (DS) rises with an increase in monochloroacetic acid (DS: 0.20–0.36) over a duration of 2 hours. The maximum degree of substitution (0.49) was achieved when the reaction time was extended from 2 to 4 hours while maintaining a constant quantity of MCA. MCA and reaction time were identified as essential parameters to enhance the degree of substitution [3].

The ratio of sodium hydroxide (NaOH) to MCA was notably significant, as the carboxymethylation process was preferentially augmented under alkaline conditions (catalyst), with MCA serving as the etherification agent. nevertheless, carboxymethylation through the modification of MCA and NaOH was constrained, especially for *Amorphophallus muelleri* glucomannan. This experiment involved modifying glucomannan obtained from *Amorphophallus muelleri* (AGM) by varying the quantity of NaOH as a catalyst and MCA as an etherification reagent to evaluate the degree of substitution of AGM. Finally, the influence of different levels of substitution on viscosity and solubility was also analyzed.

Materials and Methods

Materials and Chemicals

Amorphophallus muelleri flour was obtained from Ladang Konjak Sdn. Bhd. (Kuala Pilah, Negeri Sembilan, Malaysia). Monochloroacetic acid (MCA), sodium hydroxide (NaOH), ethanol, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), isopropyl alcohol, 3,5-dinitrosalicylic acid (DNS), and phenolphthalein were purchased from either Sigma-Aldrich or Merck. All chemicals utilized throughout this study were of analytical grade and chemically pure.

Methods

Glucomannan was extracted from *Amorphophallus muelleri* flour via acid hydrolysis employing hydrochloric acid. The DNS assay quantified the glucomannan content and its alteration through modifying various parameters, including sodium hydroxide (NaOH) and monochloroacetic acid (MCA). The extent of substitution of carboxymethylated *Amorphophallus muelleri* glucomannan was quantified through titration potentiometric techniques, which were subsequently validated by analyzing the functional groups and hydrogen composition of both unmodified and modified glucomannan. Finally, the impact of carboxymethylation on the viscosity and solubility of glucomannan was assessed to compare the alteration before and after modification.

Extraction of *Amorphophallus muelleri* glucomannan

Glucomannan was extracted from *Amorphophallus muelleri* flour using acid hydrolysis with hydrochloric acid (HCl), following the method described by Kumoro *et al.* (2016) [16]. Briefly, 1 g of flour was dissolved in 50 mL of 0.5 M HCl and stirred until fully dissolved. The mixture was subsequently stirred in a shaking water bath at 60 °C for 1 hour, followed by centrifugation at 6600×g at ambient temperature for 30 min. The supernatant, containing the solubilized glucomannan, was precipitated through the addition of 95% ethanol in a 1:1 (v/v) ratio. The precipitate was washed with ethanol and filtered through cheesecloth. The resulting undissolved solution was then collected. The entirety of coagulated glucomannan was dried overnight at 45 °C. Finally, the dried sample was ground using a mortar and sieved through a 250 mm mesh screen to obtain a uniform particle size suitable for further analysis.

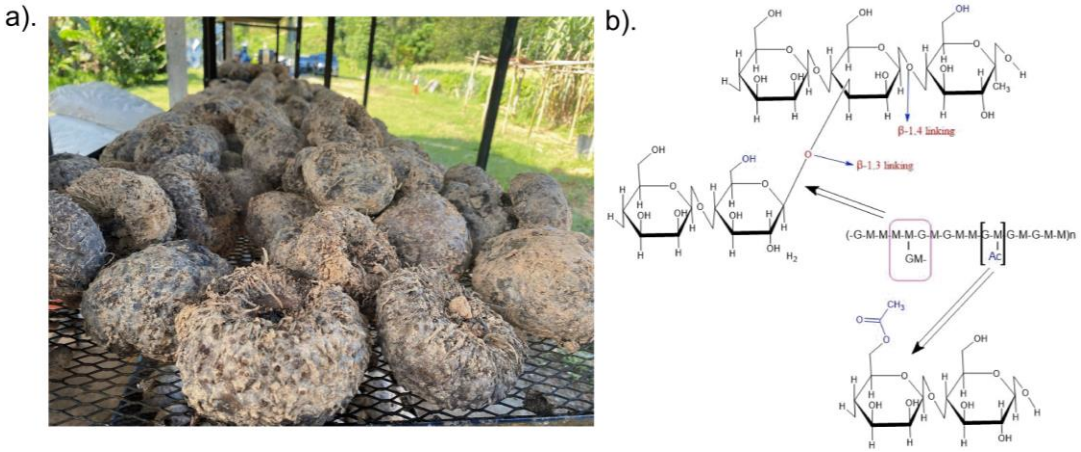

Figure 1 a) *Amorphophallus muelleri* (Ladang Konjak Sdn. Bhd., Selangor, Malaysia); b) Structure of *Amorphophallus muelleri* glucomannan

Determination of Glucomannan Content in *Amorphophallus muelleri* flour

Amorphophallus muelleri glucomannan (AGM) content was determined by utilizing the procedure described by Chua *et al*. [17]. A total of 200 mg of the glucomannan extract was dissolved in 50 mL of formic acid-sodium hydroxide buffer (pH 12) and agitated for 4 h at 25 °C. Following this, the solution was centrifuged at 2933×g for 40 min at 25 °C, and the supernatant, consisting of unhydrolyzed AGM, was collected. In order to hydrolyze the solution, 5 mL of the unhydrolyzed AGM was mixed with 2.5 mL of 3 M sulfuric acid. The solution was heated and stirred in a boiling water bath for 90 min before it was cooled to room temperature (25 °C). Subsequently, 2.5 mL of 6 M NaOH was added to neutralize the solution (i.e., hydrolyzed AGM). Distilled water was then added to bring the final volume to 25 mL, yielding the hydrolyzed glucomannan solution. Both unhydrolyzed and hydrolyzed AGM solutions were exposed to the DNS (3,5-dinitrosalicylic acid) colorimetric assay to quantify their content, with deionized water as the blank. The glucomannan content was calculated using Eq. (1):

$$\text{AGM content } (\%) = \frac{5000\text{f}(5\text{T}-\text{T}_0)}{\text{m}} \qquad \text{Eq. (1)}$$

where f is the correction factor, T is the content of glucose from AGM hydrolysate (mg), T_0 is the content of glucose from AGM extract (mg), and *m* is the mass of AGM (mg).

Preparation of Carboxymethylated *Amorphophallus muelleri* Glucomannan (CMAGM)

Carboxymethylation of *Amorphophallus muelleri* glucomannan was performed according to previous studies by Xie *et al*. and Zhu *et al*., with slight modifications [3,8]. The altered structure of glucomannan after modification is depicted in Figure 2. Firstly, 10 g of AGM flour was dissolved in 500 mL of a 10:90 (v/v) isopropyl alcohol-distilled water mixture. Subsequently, 5 g of NaOH was added to the solution and agitated at 50 °C, with a rotational speed of 400 rpm, for 1 hour. Following this, 2.5 g of monochloroacetic acid (MCA) was added to the solution, and stirring continued for 1 h under the same conditions (50 °C, 400 rpm). The solution was then precipitated using 96% (v/v) ethanol, filtered, and successively rinsed with ethanol of increasing concentrations (70-100%). The final carboxymethylated product (CMAGM) was dried in an oven at 50 °C for 3-4 h. The effect of different ratios of NaOH/MCA on the degree of substitution by carboxymethylation was evaluated. The degree of substitution (DS) was subsequently assessed as described using acid-based titration

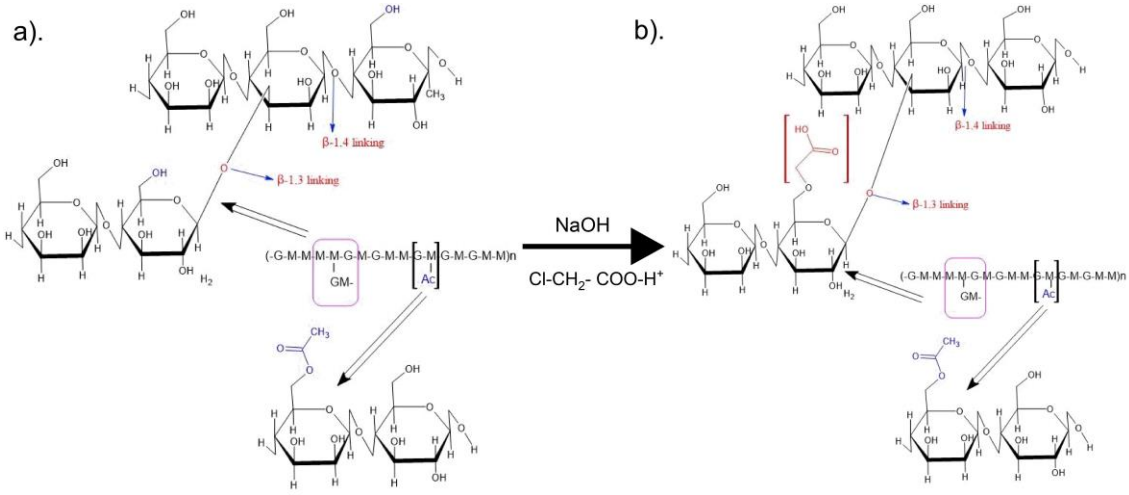

Figure 2 Carboxymethylation of *Amorphophallus muelleri* glucomannan by the etherification process at 50 °C with sodium hydroxide (NaOH) and monochloroacetic acid (MCA), a). *Amorphophallus muelleri* glucomannan (AGM) and b). carboxymethylated *Amorphophallus muelleri* glucomannan (CMAGM)

Acid-based Titration

The degree of substitution (DS) of CMAGM was determined via the acid-base titration method, following the methods described by Xie *et al*. and Zhu *et al*., with minor adjustments [3,8]. The reaction was performed by dissolving 0.5 g of CMAGM in 20 mL of 0.5 M isopropyl alcohol and HCl, followed by stirring for 5 h at room temperature. The sample was then precipitated with 80% (v/v) ethanol, filtered using a vacuum pump, and dried in an oven at ambient temperature for 3 h. Next, 0.4 g of the dried CMAGM powder was dissolved in 50 mL of 0.1 M NaOH and stirred for 1 h at 50 °C. Phenolphthalein was used as an indicator, and the endpoint was determined as the indicator's color changed from red to colorless. The carboxyl content was calculated according to the following equations (Eq. (2) and Eq. (3)):

$$DS = \frac{A\times\ 0.162}{1-A\times\ 0.058} \qquad \text{Eq. (2)}$$

$$A = \frac{C_{NaOH}\times\ V_{NaOH}-C_{HCl}\times C_{HCl}}{m} \qquad \text{Eq. (3)}$$

where A is milliequivalents of NaOH required per g of the sample, 162 is the molecular weight of the unsubstituted AGM unit, and 58 is the molecular weight of the substituting CMAGM. The C and V are the molar concentration and volume of the NaOH/HCl solution, respectively, while m is the weight of CMAGM (g).

Characterization of Carboxymethylated *Amorphophallus muelleri* Glucomannan (CMAGM)

Viscosity Measurement

The viscosities of *Amorphophallus muelleri* glucomannan (AGM) and carboxymethylated *Amorphophallus muelleri* glucomannan (CMAGM) were measured using a Brookfield DV-11+ Pro viscometer. AGM and CMAGM (different degrees of substitution) solution was prepared by dissolving and stirring 0.5% (w/v) of the glucomannan in 5 mL of distilled water at 45 °C for 90 min. The solution was centrifuged at 2300×g for 15 minutes to extract the solid material, and the supernatant was transferred to the viscometer. The viscosity was then compared to the reference data obtained from water, which served as the blank.

Solubility Analysis

Solubility of the extract AGM and CMAGM was analyzed using the procedure by Wang *et al* (2014) with slight modifications [18]. Briefly, 0.1 g of sample powder was suspended in 24.9 g of crushed ice within an ice water bath maintained at 0 °C, with vigorous agitation until the crushed ice was completely melted. The sample solution underwent centrifugation at 4030×g for 20 min. 10.00 g of the supernatant was subjected to drying in an oven at 105 °C until a consistent mass (m) was achieved. Each group was assessed in three replicates. The water solubility was determined using Equation (Eq. (4)).

$$Water\ solubility\ (\%) = \frac{2.5\times\ m}{m} \times 100 \qquad \text{Eq. (4)}$$

Functional Group Analysis

Fourier Transform Infrared Spectrophotometry (FT-IR) analysis was employed to identify the functional groups of *Amorphophallus muelleri* glucomannan (AGM) and to verify the structural modifications of AGM. This was achieved through Fourier Transform Infrared (FT-IR) (Nicolet iS5, Thermo Fisher, USA). Briefly, 30 mg of AGM flour, AGM extract, and CMAGM powder were evaluated in a KBr pellet, with measurements reported in the range of 4000 cm^{-1} to 400 cm^{-1}.

Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

Nuclear Magnetic Resonance analysis (^{1}H NMR) was utilized to determine the H composition of the AGM and modified AGM structure to confirm the modification part of the structure. Briefly, 30 mg of AGM extract and CMAGM powders were separately suspended in D_2O and subjected to ^{1}H NMR analysis, which was recorded using a Bruker 400 MHz spectrometer (Bruker, Germany).

Statistical Analysis

All statistical analyses were performed using OriginPro 2024 (OriginLab Corporation, Northampton, MA, USA). Data were analysed using one-way analysis of variance (ANOVA), paired sample t-tests, and Tukey's multiple comparison post-hoc test. Differences between various analysis groups were identified as statistically non-significant at a *P* value of > 0.05, which were represented by the same letter in the figures and tables. All the tests were performed in triplicate, and the level of significance was set at 95%.

Results and Discussion

Extraction of *Amorphophallus muelleri* Glucomannan from Flour

Amorphophallus muelleri is recognized as one of the richest sources of glucomannan, after the well-known konjac plant. It is predominantly found throughout Southeast Asia, including Malaysia. In this study, glucomannan was extracted from the *Amorphophallus muelleri* flour through acid hydrolysis using hydrochloric acid (HCl), followed by ethanol precipitation to recover the polysaccharide [16]. The main impurity in *Amorphophallus muelleri* glucomannan flour is starch. Subsequently, in terms of starch hydrolysis using the acid method, HCl was chosen as the hydrolyzing agent due to its superior performance in breaking down starch compared to sulfuric and phosphoric acids [19]. Furthermore, HCl can break all the glycosidic bonds in complex carbohydrates at a low operating cost [20]. In this study, the glucomannan content obtained was 52.4%, which was slightly lower than the 54% previously reported by Kumoro *et al*. [16]. This minor variation can be attributed to differences in cultivation methods or conditions, as well as the post-harvest treatments of the *A. muelleri* source material, which have been recorded to influence glucomannan content [21]. In addition, the multiple processing steps involved in the extraction, such as hydrolysis, filtration, evaporation, drying, grinding, and sieving, could contribute to the loss of glucomannan, resulting in a slightly reduced yield.

Several glucomannan extraction techniques have been reported in previous literature. For example, Nurlela *et al*. [22] utilized a multi-level extraction process of *A. muelleri*, where the sample was sequentially treated through the ethanol precipitation method (using 60%, 70%, and 80% ethanol), which resulted in a glucomannan yield of 66.4%. In another study, Haryani *et al*. [23,24] compared acid and enzyme hydrolysis methods for glucomannan extraction. From the studies, the acid hydrolysis method, where 0.7 M of HCl was utilized at 70 °C for 2 h with isopropyl alcohol, yielded 12.2% glucomannan, whereas enzymatic hydrolysis using α-amylase produced a higher glucomannan content of 43.4%. On the other hand, Tatirat *et al*. [25] reported that glucomannan extracted from konjac species using aluminium sulphate produced 35.4% glucomannan content. Compared to these methods, the extraction approach implemented in this study resulted in a glucomannan content (52.4%) that is comparable to, or higher than, that obtained in previous studies. Moreover, the extraction method used in this work is relatively simpler, operates at a lower temperature, and requires a shorter reaction time. Unlike enzymatic methods, which—despite being effective—entail higher operational costs and require specialized reagents and controlled conditions, this acid hydrolysis technique is more cost-efficient and scalable. Overall, the findings from this study not only validate the extraction method but also highlight the potential of *A. muelleri* flour as a valuable source of high glucomannan content.

Synthesis of Carboxymethylated *Amorphophallus muelleri* Glucomannan

Carboxymethylation is one of the etherification processes that alters the structural and functional properties of polysaccharides by introducing new carboxyl (-COOH) groups into their molecular chains [4]. In this study, carboxymethylation of *A. muelleri* glucomannan (CMAGM) was achieved using monochloroacetic acid (MCA) in the presence of NaOH as a catalyst. The incorporation of COOH into AGM is aimed at reducing its viscosity, enhancing the solubility by producing a negative charge, which leads to more hydrogen bonding, thereby broadening its application in various industries[3]. CMAGM samples with different degrees of substitution (DS) were synthesized by adjusting the MCA/NaOH ratio. The DS plays a crucial role in determining the characteristics and number of carboxymethyl groups of the glucose unit in the AGM, as OH groups in the glucose unit were converted into COOH groups [26]. The principle of carboxymethylation on polysaccharide (glucomannan) mechanism reaction, following Williamson Ether Synthesis [27] involves deprotonating the -OH groups of polysaccharides to create alkoxide groups in an alkaline solution, hence increasing their nucleophilicity. Therefore, $-CH_2COONa$ was incorporated between chloroacetic acid and polysaccharide alkoxide via an SN_2 reaction [8]. Overall, the mechanism of Williamson etherification for the polysaccharide structure was the same, for instance, in the glucomannan structure (Figure 3).

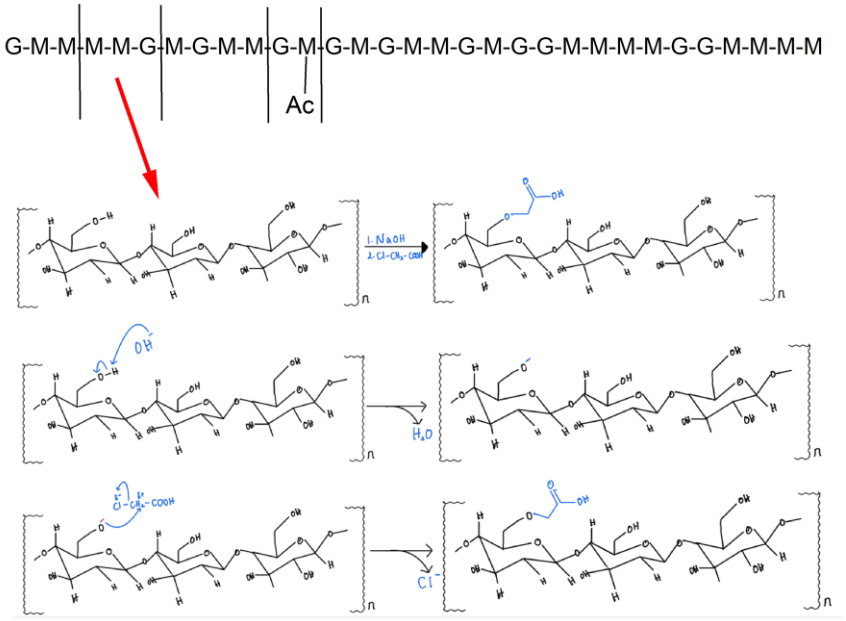

Figure 3 Reaction mechanism of Williamson Ether Synthesis on glucomannan structure

The MCA/NaOH ratio plays an important role in influencing the DS of the samples. In the etherification process, the addition of NaOH acted as a catalyst in the reaction by increasing the alkaline levels of the solution. Specifically, NaOH deprotonates the OH groups of the polysaccharide, rendering them nucleophilic and thus more reactive towards MCA, which introduces a COOH group onto the backbone. However, an excessive concentration of NaOH can be detrimental as it leads to the formation of sodium glycolate and the degradation of the AGM backbone. This occurs when MCA introduces a disproportionate number of COOH groups during the etherification process, thereby reducing the efficiency of the carboxymethylation reaction [29]. Consistent with this findings, Li *et al.* [29] reported that increasing the NaOH concentration from 4 to 6 mol/L during the carboxymethylation of corn bran polysaccharide increased the degree of substitution (DS) to 0.53; however, a further increase to 8 mol/L resulted in a decline in DS to 0.46. In addition to NaOH concentration, polymer solubility under alkaline conditions also influences reaction efficiency. In alkaline conditions, only lower concentrations of AGM (< 0.5% w/v) are soluble as higher concentrations of AGM can result in gelatinization that hinders the etherification process [30]. To address this limitation, co-solvents such as alcohol are introduced in order to facilitate the accessibility of the etherifying agent. Overall, these findings underscore importance of maintaining a balanced MCA/NaOH ratio to achieve an optimal DS.

Table 1 Preparation conditions of carboxymethylated *Amorphophallus muelleri* and the degree of substitution

	Preparation condition						
Sample	**AGM (%)**	**MCA (%)**	**NaOH (%)**	**10% Isopropyl Alcohol (mL)**	**Activation with NaOH (h)**	**Reaction Time with MCA (h)**	**Degree of Substitution (DS)**
Extract	1	-	-	-	-	-	-
CMAGM A	1	2	4	250	1	1	0.577 ± 0.033^{a}
CMAGM B	1	4	2	250	1	1	0.335 ± 0.005^{cd}
CMAGM C	1	4	4	250	1	1	0.459 ± 0.015^{b}
CMAGM D	1	6	4	250	1	1	0.405 ± 0.008^{bc}
CMAGM E	1	8	4	250	1	1	0.308 ± 0.050^{d}

*All experiments were conducted in triplicate. The error bar indicates the standard deviation. The significant differences of the mean values are denoted by different letters when $P < 0.05$ by the ANOVA and Tukey's test.

Hence, in this study, the optimal MCA/NaOH ratio for the CMAGM was investigated to achieve the highest degree of substitution (DS). As summarized in Table 1, the maximum DS (0.577) was attained at MCA/NaOH concentration of 2:4% (w/v). It was observed that an equivalent quantity of MCA (CMAGM B and C at a concentration of 4%) combined with an increased NaOH concentration from 2 to 4% led to an elevation in the degree of substitution (DS) from 0.335 to 0.459, indicating that a higher alkaline condition enhances the efficiency of the carboxymethylation reaction.

Conversely, maintaining a NaOH concentration of 4% while increasing the MCA from 2 to 8% resulted in a mere reduction of the DS from 0.577 to 0.308. This suggests that excess MCA does not promote further substitution, possibly due to the limited accessibility of the reactive hydroxyl group with the same amount of AGM and NaOH. This indicate the formation of the intermediate AGM–ONa complex, produced through the initial reaction between AGM and NaOH may be the rate-limiting step in this process [9]. The result contrasts with earlier findings on the carboxymethylation of konjac glucomannan (CMKGM), where an increase in MCA generally leads to a higher DS [2]. This discrepancy may be attributed to the lower initial concentration of AGM used in the present study compared to the previous study, which likely altered the reaction dynamics.

Notably, when the NaOH concentration was decreased from 4 to 2% with adjusting the ratio of MCA/NaOH from 2:4% w/v to 4:2 % (w/v), the degree of substitution (DS) of CMAGM substantially dropped from 0.577 to 0.335, indicating that 4% NaOH and 2% MCA are adequate to promote the reaction between MCA and AGM. This is because the concentration of NaOH is below 4%, resulting in

a reduction of the DS for CMAGM. This observation highlights the critical role of NaOH in activating and catalyzing the etherification process with by MCA as insufficient alkaline conditions hinder the formation of alkoxide intermediated, thereby reducing the efficiency of MCA to substitute the structure of AGM.

Viscosity and Solubility

Table 2 illustrates the viscosity and solubility corresponding to various degrees of substitution in the carboxymethylation of *Amorphophallus muelleri* glucomannan (CMAGM). It was observed that an increase in DS corresponded to a reduction in the viscosity of CMAGM (Table 2). An increase in DS resulted in a progressive decrease in viscosity accompanied by a marked enhancement in solubility. This observation is consistent with previous findings by Wang *et al*. [2], who reported that carboxymethylation, by using NaOH as the activator, suppresses the intermolecular hydrogen bonding in aqueous solutions of KGM. In the present study, the weakened hydrogen bonding may have counteracted the viscosity-enhancing effects of deacetylation, resulting in the decreased viscosity and increased solubility of the AGM. Generally, the major functional group that contributes to the solubility of glucomannan is the acetyl group, which can be lost during modification under alkaline conditions, leading to the entanglement and aggregation of the molecule [31]. However, at higher DS, the introduction of carboxymethyl groups into the molecules causes the multi-branch structures to interact with other molecules, improving the hydrophilicity and polymer water interaction, causing an increase in the solubility while diminishing the viscosity. This demonstrated that DS plays a crucial role in promoting electrostatic repulsion between polymer chains, facilitating chain separation, and imparting viscosity and solubility. Overall, the reduction in viscosity and augmentation of solubility broaden the applicability of glucomannan, making it suitable for a range of applications where tailored rheological properties are required.

Table 2 Degree of substitution and its influence on the viscosity and solubility

Sample	DS	Viscosity (cP)	Shear Stress (D/cm^2)	Shear Rate (1/sec)	Solubility (%)
Extract AGM		2.63	1.84	70	0.50
CMAGM E	0.308	2.20	1.54	70	21.25
CMAGM B	0.335	2.17	1.52	70	24.00
CMAGM D	0.405	1.84	1.29	70	25.00
CMAGM C	0.459	1.68	1.17	70	26.75
CMAGM A	0.577	1.48	1.04	70	35.35

Characterization of *Amorphophallus muelleri* Glucomannan (AGM) and Carboxymethylated *Amorphophallus muelleri* Glucomannan (CMAGM)

Functional Group Analysis

Figure 3 presents the FT-IR spectra of crude AGM, AGM extract, and CMAGM powders. All three samples (i.e., crude AGM, AGM extract, and CMAGM) exhibited a broad absorption band in the range of 3600-3280 cm^{-1}, attributed to OH stretching vibrations, as well as a peak at 2932 cm^{-1} corresponding to the stretching vibrations of methylene (CH_2) groups. In addition, another peak was observed at 1009 cm^{-1}, representing CO stretching vibrations, which is characteristic of polysaccharide structures. In crude AGM flour, an absorption band at 1617 cm^{-1} was detected, indicating the presence of strong intermolecular hydrogen bonding between polysaccharides [2]. This band was noticeably less prevalent in the AGM extract, suggesting that the extraction process may have altered the hydrogen bonding networks and concurrently removed various impurities that may interfere with functional group interactions. As a result, a distinct absorption band emerged at 1742 cm^{-1} in the AGM extract spectrum, indicating the presence of the acetyl group (CH_2COOCH_3) in the glucomannan extracted from AGM.

In contrast, the characteristic acetyl-related band at 1742 cm^{-1} was not detected in the CMAGM spectrum. The absence of the band is attributed to the deacetylation that occurred under harsh alkaline conditions employed during the carboxymethylation process. These conditions appear to disrupt the intramolecular hydrogen bonding responsible for the acetyl-associated vibration, leading to the disappearance of this spectral feature. Instead, new bands appeared in the region of 1587–1369 cm^{-1}, corresponding to the symmetric and asymmetric stretching vibrations of the carboxylate (COO^-) groups introduced via carboxymethylation. This structural transformation is consistent with previous findings on konjac glucomannan, which reported the replacement of the acetyl groups by carboxymethyl groups during the carboxymethylation process [9]. Subsequently, the successful substitution suggests effective

carboxymethylation of AGM under the applied reaction conditions.

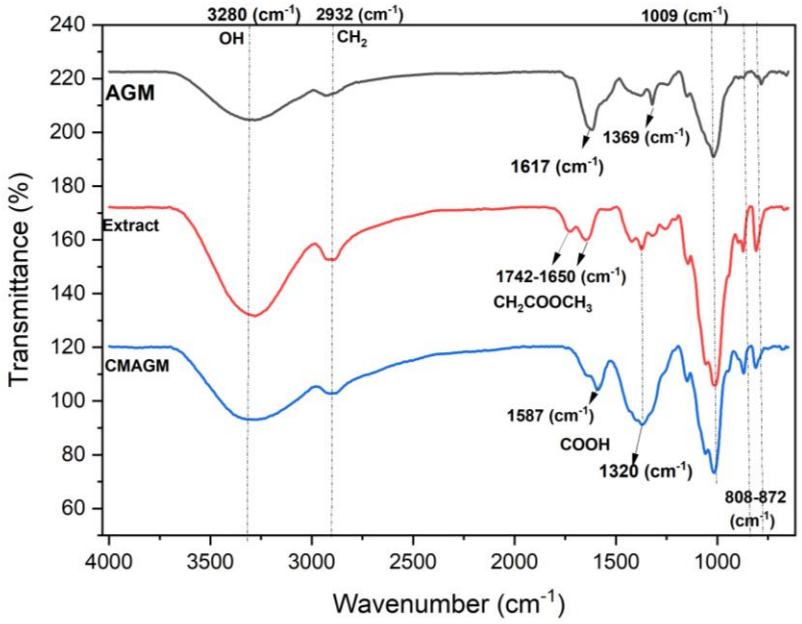

Figure 4 The FT-IR spectra of CMAGM, AGM extract, and AGM

Moreover, the modification of AGM significantly altered the hydrogen bonding network within the polymer matrix, as indicated by changes in the intensity and broadness of the spectra regions, which may have affected the interaction of the biopolymer [7]. This was also corroborated by the FT-IR spectra, where the OH peak in the CMAGM appeared less intense than in the extracted AGM, indicating a decrease in hydrogen bonding. Similarly, this change is likely a consequence of the alkaline conditions during carboxymethylation that influenced the intermolecular interactions of the biopolymer [7],[26]. Despite these chemical modifications, both AGM and CMAGM displayed characteristic absorption peaks in the range of 872–808 cm^{-1}, which are attributed to the β-glycosidic linkages associated with D-mannose residues in the glucomannan backbone [32]. The presence of these peaks confirms the preservation of the core polysaccharide structure despite chemical modifications. Overall, the observed spectral changes confirm the successful extraction, deacetylation, and carboxymethylation of AGM, demonstrating the structural transformations induced during the preparation of CMAGM and their impact on the functional group composition and molecular interactions within the biopolymer.

^{1}H Nuclear Magnetic Resonance (NMR) Analysis

The ^{1}H NMR spectra of the extracted AGM and CMAGM are presented in Figure 4. A new signal appearing at δ = 3.3-4.1 δ ppm in the CMAGM spectrum corresponds to the methylene protons introduced during carboxymethyl modification under alkaline conditions. This result aligns with previous findings that attributed the chemical shift range to methylene protons in carboxymethylated polysaccharides [33]. Additionally, a distinct signal at δ = 8.057 ppm in the CMAGM spectrum corresponds to the proton of the COOH group. Subsequently, the presence of these two characteristic signals further confirms the successful carboxymethylation of AGM. Furthermore, both AGM extract and CMAGM exhibited a chemical shift in the range of δ = 3.0–4.5 ppm, which corresponds to the H2-H6 of glucose and mannose residues [26]. A separate signal observed between δ = 4.5–5.4 ppm was attributed to the H1 of monomeric glucose and mannose, consistent with the structural features of the glucomannan backbone [26]. Lastly, a signal at δ = 1.9–2.02 ppm, observed in both AGM and CMAGM spectra, was assigned to the acetyl groups. Notably, the signal intensity was higher in the CMAGM spectrum compared to the AGM spectrum, which may be due to the structural rearrangements under the alkaline modification conditions. In summary, the ^{1}H NMR results support the FT-IR findings and confirm the structural transformation of AGM through carboxymethylation. The appearance of characteristic chemical shifts for carboxymethyl and carboxylic acid groups, alongside the preserved signals of the key polysaccharide structure, demonstrates the successful chemical modification and structural integrity of the glucomannan backbone.

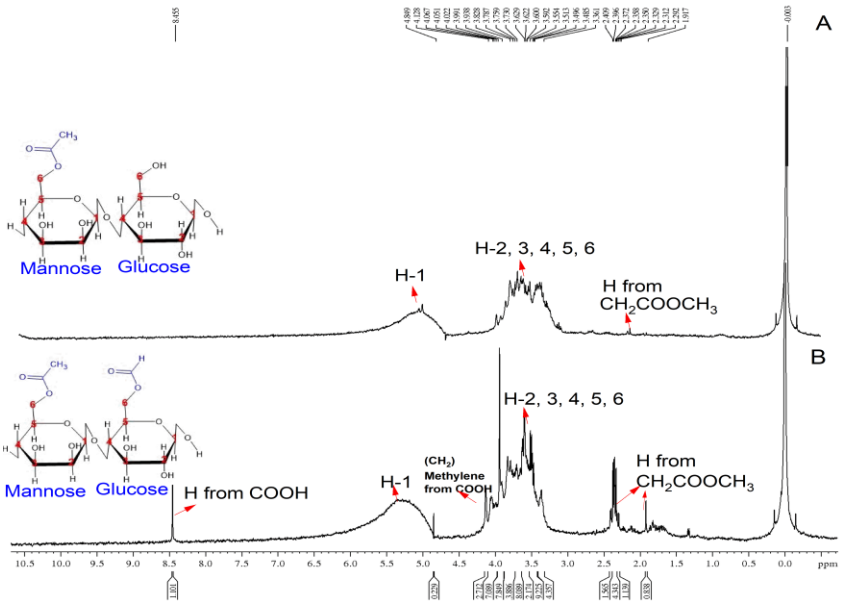

Figure 5 Proton (H) nuclear magnetic resonance (NMR) spectra of AGM extract and CMAGM. (a) 1H NMR spectra of AGM extract (b) 1H NMR spectra of CMAGM

Conclusion

Glucomannan derived from *Amorphophallus muelleri* (AGM) was effectively subjected to the carboxymethylation procedure. The resulting carboxymethylated AGM (CMAGM) introduced carboxyl groups into the polymer backbone. Different degree of substitution was influenced by the introduction of the carboxyl group into the structure of glucomannan. The degree of substitution (DS) of the carboxymethylated *Amorphophallus muelleri* glucomannan (CMAGM) improved as the quantity of monochloroacetic acid (MCA) diminished, suggesting that the initial reaction between AGM and sodium hydroxide (NaOH) could be the rate-limiting step in this process. An increase in the degree of substitution reduces viscosity while enhancing the solubility of the AGM. This is because an increased degree of substitution corresponds to a greater number of COOH groups incorporated into the structure, thereby enhancing hydrogen bonding interactions with water.

Conflict of Interest

The authors declare that they have no relevant competing financial interests or non-financial interests that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors gratefully acknowledge the financial support from Ministry of Higher Education, Malaysia and Universiti Teknologi Malaysia under the UTM Fundamental Research (Q.J130000.3846.23H31), Geran Penyelidikan Hi-Tech(F4+) 2024 (Q.J130000.4609.00Q37), as well as Professional Development Research University Grant (R.J130000.7113.07E91), where Nur Aizura Mat Alewi is a Post Doctoral Fellow of Universiti Teknologi Malaysia under the Post Doctoral Fellowship Scheme.

Reference

[1] Xu, R., Zheng, X., Chen, C., Li, M., Li, J., Zhou, H., *et al*. (2024). Effects of different substrates on the growth and yield of *Amorphophallus muelleri*. *Heliyon, 10*, e31501. https://doi.org/10.1016/j.heliyon.2024.e31501

[2] Wang, L. H., Huang, G. Q., Xu, T. C., & Xiao, J. X. (2019). Characterization of carboxymethylated

konjac glucomannan for potential application in colon-targeted delivery. *Food Hydrocolloids, 94*, 354–362. https://doi.org/10.1016/j.foodhyd.2019.03.045.
[3] Zhu, C., Zhang, X., Gan, J., Geng, D., Bian, X., Cheng, Y., *et al*. (2023). A pH-sensitive hydrogel based on carboxymethylated konjac glucomannan crosslinked by sodium trimetaphosphate: Synthesis, characterization, swelling behavior and controlled drug release. *International Journal of Biological Macromolecules, 232*. https://doi.org/10.1016/j.ijbiomac.2023.123392.
[4] Hu, Q., Huang, G., & Huang, H. (2025). Extraction, structure, activity and application of konjac glucomannan. *Ultrasonics Sonochemistry, 116*. https://doi.org/10.1016/j.ultsonch.2025.107315.
[5] Hikmat, R., Resdiana, R., & Tasfiyati, A. N. (2021). Carboxymethyl glucomannan from *Amorphophallus oncophyllus* as an alternative heavy metal removal from wastewater: Adsorption of cadmium and zinc. *Molekul, 16*, 57–66. https://doi.org/10.20884/1.jm.2021.16.1.682.
[6] Aanisah, N., Wardhana, Y. W., Chaerunisaa, A. Y., & Budiman, A. (2022). Review on modification of glucomannan as an excipient in solid dosage forms. *Polymers (Basel), 14*, 2550. https://doi.org/10.3390/polym14132550.
[7] Lohani, A., Saxena, R., & Khan, S., Mascarenhas-Melo, F. (2024). pH-responsive IPN beads of carboxymethyl konjac glucomannan and sodium carboxymethyl cellulose as a controlled release carrier for ibuprofen. *International Journal of Biological Macromolecules, 278*, 134676. https://doi.org/10.1016/j.ijbiomac.2024.134676.
[8] Xie, Y., Yi, Z. X., Wang, J. X., Hou, T. G., & Jiang, Q. (2018). Carboxymethyl konjac glucomannan-crosslinked chitosan sponges for wound dressing. *International Journal of Biological Macromolecules, 112*, 1225–1233. https://doi.org/10.1016/j.ijbiomac.2018.02.075.
[9] Ding, X., Zhang, L., Jiang, C., Liu, S., Li, H., Xi, J., *et al*. (2024). Building covalent crosslinks of carboxymethyl konjac glucomannan with boronic ester bonds for fabricating multimodal hydrogel sensor. *International Journal of Biological Macromolecules, 277*, 134286. https://doi.org/10.1016/j.ijbiomac.2024.134286.
[10] Niu, C., Wu, W., Wang, Z., Li, S., & Wang, J. (2007). Adsorption of heavy metal ions from aqueous solution by crosslinked carboxymethyl konjac glucomannan. *Journal of Hazardous Materials, 141*, 209–214. https://doi.org/10.1016/j.jhazmat.2006.06.114.
[11] Liang, L., Lin, X., Liu, Y., Sun, S., Chu, H., Chen, Y., *et al*. (2020). Carboxymethyl konjac glucomannan mechanically reinforcing gellan gum microspheres for uranium removal. *International Journal of Biological Macromolecules, 145*, 535–546. https://doi.org/10.1016/j.ijbiomac.2019.12.188.
[12] Wu, C., Sun, J., Jiang, H., Li, Y., & Pang, J. (2021). Construction of carboxymethyl konjac glucomannan/chitosan complex nanogels as potential delivery vehicles for curcumin. *Food Chemistry, 362*, 130242. https://doi.org/10.1016/j.foodchem.2021.130242.
[13] Xiao, M., Jiang, M., Wu, K., Yang, H., Ni, X., Yan, W., *et al*. (2017). Investigation on curdlan dissociation by heating in water. *Food Hydrocolloids, 70*, 57–64. https://doi.org/10.1016/j.foodhyd.2017.03.018
[14] Guo, L., Yokoyama, W., Chen, L., Liu, F., Chen, M., & Zhong, F. (2021). Characterization and physicochemical properties analysis of konjac glucomannan: Implications for structure-properties relationships. *Food Hydrocolloids, 120*, 106818. https://doi.org/10.1016/j.foodhyd.2021.106818.
[15] Aprilia, V., Murdiati, A., Hastuti, P., & Harmayani, E. (2017). Carboxymethylation of glucomannan from porang tuber (*Amorphophallus oncophyllus*) and the physicochemical properties of the product. *Pakistan Journal of Nutrition, 16*, 835–842. https://doi.org/10.3923/pjn.2017.835.842.
[16] Kumoro, A. C., Yuganta, T. H. A., Ratnawati, R., & Retnowati, D. S. (2016). Effect of catalyst concentration and reaction time on the extraction of glucomannan from porang (*Amorphophallus oncophyllus*) flour via acid hydrolysis. *IOP Conference Series: Materials Science and Engineering, 162*, 012020. https://doi.org/10.1088/1757-899X/162/1/012020.
[17] Chua, M., Chan, K., Hocking, T. J., Williams, P. A., Perry, C. J., & Baldwin, T. C. (2012). Methodologies for the extraction and analysis of konjac glucomannan from corms of *Amorphophallus konjac* K. Koch. *Carbohydrate Polymers, 87*, 2202–2210. https://doi.org/10.1016/j.carbpol.2011.10.053.
[18] Wang, S., Wu, X., Wang, Y., Li, Y., Wang, L., Chen, Y., *et al*. (2014). Dissolution behavior of deacetylated konjac glucomannan in aqueous potassium thiocyanate solution at low temperature. *RSC Advances, 4*, 21918–21923. https://doi.org/10.1039/c4ra01491j.
[19] Kumoro, A., Yuganta, T., Retnowati, D., & Ratnawati, R. (2018). Acid hydrolysis and ethanol precipitation for glucomannan extraction from crude porang (*Amorphophallus oncophyllus*) tuber flour. *Chemistry & Chemical Technology, 12*, 101–108. https://doi.org/10.23939/chcht12.01.101.
[20] Plermjai, K., Mekprasart, W., Boonyarattanakalin, S., & Boonyarattanakalin, K. (2023). Enhancing saccharide extraction from konjac by microwave-assisted acid hydrolysis. *Thai Journal of Nano Science and Technology, 8*, 11–17. https://doi.org/10.55003/tjnn8120232.
[21] Harijati, N., Mastuti, R., Chairiyah, N., Roosdiana, B., & Rohmawati, S. A. (2018). Effects of seeding material age, storage time, and tuber tissue zone on glucomannan content of *Amorphophallus muelleri* Blume. *International Journal of Plant Biology, 9*, 34–38. https://doi.org/10.4081/pb.2018.7626.
[22] Nurlela, N., Ariesta, N., Santosa, E., & Muhandri, T. (2022). Physicochemical properties of glucomannan isolated from fresh tubers of *Amorphophallus muelleri* Blume by a multilevel extraction method. *Food Research, 6*, 345–353. https://doi.org/10.26656/fr.2017.6(4).580.
[23] Haryani, K., Prasetyaningrum, A., Handayani, A., Kevin, F., & Nadya, T. (2023). Extraction of glucomannan from porang (*Amorphophallus oncophyllus*) flour using enzymatic hydrolysis pretreatment. *International Journal of Chemical and Biological Sciences, 24*, 362–368.
[24] Haryani, K., Handayani, N. A., & Alkautsar, A. A. (2024). Purification of glucomannan from porang (*Amorphophallus oncophyllus*) tuber flour through hydrolysis of hydrochloric acid. *International Journal of Chemical and Biological Sciences, 26*, 110–119.
[25] Tatirat, O., & Charoenrein, S. (2011). Physicochemical properties of konjac glucomannan extracted

from konjac flour by a simple centrifugation process. *LWT – Food Science and Technology, 44*, 2059–2063. https://doi.org/10.1016/j.lwt.2011.07.019.
[26] Guo, Y., Wu, M., Li, R., Cai, Z., & Zhang, H. (2022). Thermostable physically crosslinked cryogel from carboxymethylated konjac glucomannan fabricated by freeze-thawing. *Food Hydrocolloids, 122*. https://doi.org/10.1016/j.foodhyd.2021.107103.
[27] Wade, L. G. (2013). *Organic Chemistry* (8th ed.). Pearson Education.
[28] Xie, L., Shen, M., Wang, Z., & Xie, J. (2021). Structure, function and food applications of carboxymethylated polysaccharides: A comprehensive review. *Trends in Food Science & Technology, 118*, 539–557. https://doi.org/10.1016/j.tifs.2021.09.016.
[29] Li, J., Shang, W., Si, X., Bu, D., Strappe, P., Zhou, Z., *et al*. (2017). Carboxymethylation of corn bran polysaccharide and its bioactive property. *International Journal of Food Science & Technology, 52*, 1176–1184. https://doi.org/10.1111/ijfs.13382.
[30] Kobayashi, S., Tsujihata, S., Hibi, N., & Tsukamoto, Y. (2002). Preparation and rheological characterization of carboxymethyl konjac glucomannan. *Food Hydrocolloids, 16*, 289–294. https://doi.org/10.1016/S0268-005X(01)00101-1.
[31] Xiao, M., Dai, S., Wang, L., Ni, X., Yan, W., Fang, Y., *et al*. (2015). Carboxymethyl modification of konjac glucomannan affects water binding properties. *Carbohydrate Polymers, 130*, 1–8. https://doi.org/10.1016/j.carbpol.2015.05.001
[32] Cao, G., Bu, N., Zeng, T., Sun, R., Mu, R., Pang, J., *et al*. (2022). Development of pH-responsive konjac glucomannan/pullulan films incorporated with acai berry extract to monitor fish freshness. *International Journal of Biological Macromolecules, 219*, 897–906. https://doi.org/10.1016/j.ijbiomac.2022.08.030
[33] Chen, N., Zhu, P., Du, T., Han, K., Wang, D., Ye, J., *et al*. (2018). Preparation of modified konjac glucomannan nanoparticles and their application as vaccine adjuvants to promote ovalbumin-induced immune response in mice. *Pharmaceutical Research, 35*, 1–13. https://doi.org/10.1007/s11095-018-2381-x.