



Performances of Amphiphilic Glucomannan Produced by Combination Methods of Ultrasonication, Deacetylation, and Carboxymethylation Heterogeneously

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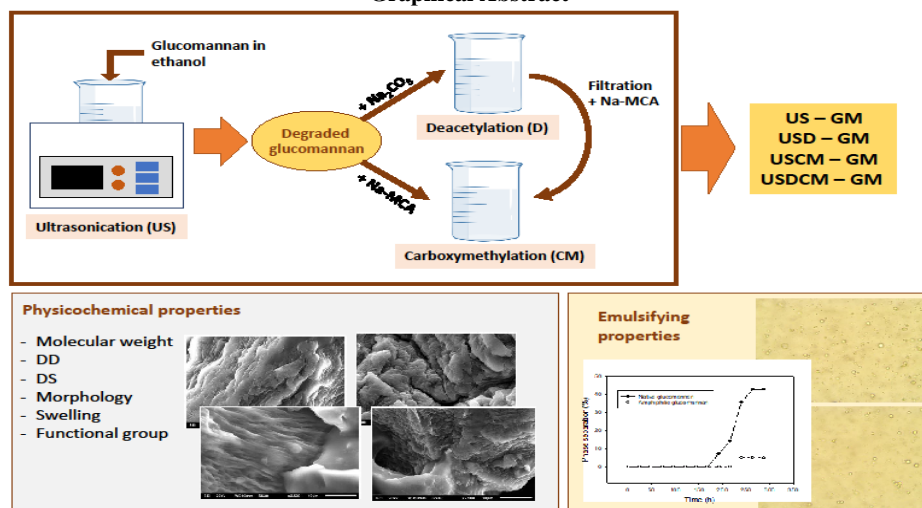
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ABSTRACT

Glucomannan carboxymethylation is conducted to increase its hydrophobic properties and expand its interaction with hydrophobic compounds. However, glucomannan has high molecular weight and long polysaccharide chain which prevented the modification of its amphiphilic properties. This study aimed to examine the effect of molecular weight, deacetylation, and carboxymethylation on glucomannan properties. Performance of the modified glucomannan to stabilize oil in water (o/w) emulsion was also studied. Ultrasonication was applied to glucomannan at 40 kHz for 15-45 min to obtain various molecular weights. Sodium carbonate (Na_2CO_3) and sodium monochloroacetate were used as deacetylation and carboxymethylation agents, respectively. The results show that decrease molecular weight supported the deacetylation and the carboxymethylation process on attaching the hydrophobic groups to the glucomannan chain, hence, lowering the hydrophilic properties and swelling degree of glucomannan. Structural and morphological changes of glucomannan after modifications were confirmed from the IR spectra and SEM images. Excellent performance of the amphiphilic glucomannan on stabilizing o/w emulsion was observed as only ~5% phase separation occurred after 300 h of storage in ambient conditions. Hence, ultrasonication is proposed as a suitable preliminary treatment for amphiphilic glucomannan production.

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Graphical Abstract



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NOMENCLATURE			
C	Concentration (g/l)	N	Normality concentration (N)
CM	Carboxymethylation	n	Group content
D	Deacetylation	t	Time (h or min or s)
DD	Degree of deacetylation	US	Ultrasonication
DS	Degree of substitution	V	Titrant volume (ml)
K	Mark-Houwink constant (ml/g)	Greek Symbols	
M	Molarity concentration (M)	η	Viscosity (Pa.s)
m	Material mass (g)	ω	Acetyl content
Mw	Molecular weight	α	Mark-Houwink constant

1. INTRODUCTION

Glucomanan, a polysaccharide extracted from *Amorphophallus sp.*, is a neutral polysaccharide that is highly soluble in water. Apart from its high viscosity, glucomanan is also known for its gel-forming ability and low calories. Other than applied as a food ingredient, glucomanan is also used in the pharmaceutical, cosmetic, and chemical industries (1). However, some potential applications of glucomanan are limited by its high solubility and water absorption index which is up as high as 100 g water/g sample (2). The strong hydrophilicity of glucomanan led to poor water resistance and water barrier properties in its films which seriously hindered its practical applications (3).

For broader glucomanan applications, several methods have been developed to convert glucomanan into an amphiphilic compound which is capable of binding hydrophilic and lipophilic compounds (4). Molecular structure of material, such as the presence of long-chain hydrocarbon or short polymer chain, contributes on the amphiphilic properties (5). Hence, the amphiphilic glucomanan could be formed through a functionalization process by designing an attachment of functional groups or moieties to enhance its use (6). This amphiphilic polysaccharide has the potential to be applied in the plastics industry, especially in the production of biodegradable films, resins, and coatings for the pharmaceutical and food industries (5). The modified glucomanan could also be performed for example as a stabilizer in o/w emulsion and encapsulant oil-base bioactive compound (7).

It has been reported that carboxymethylation decreased the water-binding activity of starch and curdlan (8, 9). Carboxymethyl modification also successfully reduced solubility and water absorption of glucomanan, which further altered its surface properties (10, 11). In this modification, the hydrophilic groups of glucomanan, i.e. hydroxyl groups, are substituted with hydrophobic groups of monochloroacetic compounds (10). The modification consists of two steps of reactions: (1) deacetylation/alkalization and (2) carboxymethylation itself. Deacetylation or alkylation is performed to eliminate acetyl groups of glucomanan under an alkaline environment (12). This replacement reduces glucomanan steric hindrance and increases its

intramolecular interaction; thus, leading to more active sites to be involved in the subsequent carboxymethylation reaction (13). The deacetylation was performed under an alkaline environment using NaOH, KOH, Ca(OH)₂, or Na₂CO₃ (14). Among those alkalis, Na₂CO₃ is safer to be consumed and does not disturb human body metabolism (15).

Xiao et al. (10) reported that various degrees of substitution (DS) using monochloroacetic acid that increase hydrophobic properties of glucomanan. In that work, the DS was obtained by varying reaction temperature, concentration of sodium, and reaction time. Moreover, the effectivity of the carboxymethylation process is influenced by the carboxymethyl reagents, temperature, duration, solvent, and polymer type (16).

Other than the presence of hydrophilic and hydrophobic groups, the length of each the groups also determines the performance of amphiphilic materials as surfactants. Day et al. (17) found an emulsion is more stable when using a surfactant with less content of the hydrophilic blocks.

Glucomanan has a molecular weight of up to 1.044×10^6 Da which makes it as one of the highest-viscosity polysaccharides (1% = 15 Pa.s) (18). This condition and its ability to form gel prevent the glucomanan to interact with other molecules during modification. To obtain a high yield of modified glucomanan, decreasing the viscosity by lowering glucomanan concentration is not an option. Hence, glucomanan requires to be degraded prior to the amphiphilic modification. Degradation of glucomanan has been conducted through chemical, physical, and biological treatments to shorten the polymer chain, thus lowering its molecular weight (19). Among those methods, physical treatment was the preferable method to decrease the molecular weight due to safety reasons either for the process, the products or for the environment (20). Ultrasound, as one of the physical treatments, produced shear forces formed by the microbubble explosion, which is powerful enough to break the polysaccharide chain and reduce molecular weight of the compound (21, 22). Lower molecular weight of glucomanan has a lower viscosity which allows it to have more mobility hence higher chances of colliding with other molecules to pursue the reaction (23).

The significance of molecular weight on the emulsifying properties of carboxymethylated glucomannan remains limited. Hence, this research focused on studying the effect of molecular weight on the amphiphilic properties of modified glucomannan. Various molecular weights were obtained by controlling duration of the ultrasonication treatment. To modify the amphiphilic glucomannan, Na₂CO₃ was applied in deacetylation process while carboxymethylation was carried out using sodium monochloroacetate. The modifications were conducted subsequently under ethanol solution. The alter of glucomannan properties were observed, i.e. molecular weight, degree of deacetylation, degree of substitution, swelling degree, functional group, and surface morphology. The ability of native and modified glucomannan to stabilize o/w emulsion was also compared.

2. MATERIALS AND METHOD

2. 1. Materials Glucomannan of NOW Foods (Bloomington, Illinois, US) was used as it is, with 99.97% purity and weight average molecular weight (Mw) of 901,175 Da determined by the GPC method (24). Sodium monochloroacetate, Na₂CO₃, ethanol, phenolphthalein indicator, and other supporting materials were in pro-analyst standard.

2. 2. Glucomannan Modification In this study, glucomannan was treated physically and chemically. Firstly, glucomannan was physically degraded using ultrasonication to obtain various viscosity-average molecular weights (Mw). Subsequently, chemical modification to obtain the amphiphilic glucomannan was conducted using a combination of deacetylation and carboxymethylation. The detailed procedure is explained in the following sections.

2. 2. 1. Glucomannan Degradation Glucomannan degradation was conducted using sonication power in a heterogeneous system. Glucomannan (20 g) was placed in Erlenmeyer, together with ethanol solution (80%, 100 ml). The Erlenmeyer was closed using perforated lid and was put in an ultrasonic cleaner (Krisbow Indonesia, West Jakarta, Jakarta, Indonesia) which was filled with water and set at 40 kHz. The ultrasonication was carried out for 15, 30, and 45 min. The ultrasonicated glucomannan was separated from the ethanol by filtration and dried at room temperature for 24 h.

2. 2. 2. Preparation of an Amphiphilic Glucomannan The amphiphilic sample was prepared using deacetylation and carboxymethylation methods (14, 25). Deacetylation was carried out by dispersing the sonicated glucomannan (5 g) in ethanol

solution (80%, 100 ml) and mixing it with 100 ml 0.4M Na₂CO₃ under constant stirring. After 24 h of the reaction, glucomannan was filtered and washed two times using 50 ml of 65% ethanol and 96% ethanol, subsequently. The deacetylated glucomannan was dried at room temperature for 24 h.

Carboxymethylation was conducted by mixing the sample (5 g) with sodium monochloroacetate (3.5 g) in ethanol solution (80%, 50 ml) at 60°C for 120 min. The glucomannan was filtered and washed using 100 ml of 96% ethanol. The amphiphilic glucomannan was then dried at room temperature for 24 h.

2. 3. Physicochemical and Stabilizing Properties

The native and modified glucomannan were analyzed for their physicochemical properties, i.e. molecular weight, degree of deacetylation (DD), degree of substitution (DS), swelling power, functional group, and morphology. The emulsifying performance of native and modified glucomannan was also observed. The detailed procedure of the determinations is written below.

2. 3. 1. Viscosity-average Molecular Weight The viscosity-average molecular weight (Mw) of glucomannan was determined using the viscometry method (20). Flow time (t₁) of the sample solution (0.1 – 0.5 g/l) in Cannon Fenske viscometer, size 100 (Schott AG, 102 Mainz, Rhineland-Palatinate, Germany) was recorded, compared to the flow time of distilled water as a solvent (t₀). Relative viscosity (η_r), specific viscosity (η_{sp}), reduction viscosity (η_{rd}), and inherent viscosity (η_{in}) of the samples were calculated using Equation 1- 4, respectively.

$$\eta_r = \frac{t_1}{t_0} \quad (1)$$

$$\eta_{sp} = \frac{t_1 - t_0}{t_0} = \eta_r - 1 \quad (2)$$

$$\eta_{rd} = \frac{\eta_{sp}}{c} \quad (3)$$

$$\eta_{in} = \frac{\ln(\eta_{rd})}{c} \quad (4)$$

The reduction viscosity and the inherent viscosity were then plotted in a graph. The intrinsic viscosity (η) was obtained by averaging the intercepts of both graphs. The average Mw of glucomannan was calculated using intrinsic viscosity value using Equation 5, with K=0.000506 ml/g and α=0.754 as Mark-Houwink constants (26).

$$M_w = \left(\frac{\eta}{K}\right)^{1/\alpha} \quad (5)$$

2. 3. 2. Degree of Deacetylation The back titration method was used to determine the degree of deacetylation (DD). Sample (1 g) was dispersed in

ethanol solution (75%, 10 ml) at 50°C. After 30 min, 1 ml of 0.5 M KOH solution was added. Constant stirring was applied during the analysis. After 24 h, the mixture was titrated with 0.1 M HCl solution using phenolphthalein as an indicator. Titrant volume was recorded and used for calculating the DD using Equations 6-8.

$$\omega_0 = \frac{(V_2 - V_0) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100\% \quad (6)$$

$$\omega = \frac{(V_2 - V_1) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100\% \quad (7)$$

$$DD = \frac{\omega_0 - \omega}{\omega_0} \times 100\% \quad (8)$$

where V_2 , V_1 , and V_0 are the volume of HCl for the blank titration, the deacetylated glucomannan, and the native glucomannan, respectively. N_{HCl} is the concentration of HCl, $M_{acetyl}=43$ g/mol, and m_s is the mass of the sample to be titrated. Meanwhile, ω_0 and ω are the amounts of acetyl in the native glucomannan and in the deacetylated glucomannan, respectively.

2. 3. 3. Degree of Substitution The determination of the degree of substitution (DS) followed the method of Distantina et al. (27). Sample (1 g) was dispersed in a HCl solution (1.8 M, 10 ml) and stirred for 30 min. After filtration, the solid was washed using 96% ethanol solution and dried in ambient conditions. The dried glucomannan was dispersed in NaOH solution (0.2 M, 20 ml) and diluted to 100 ml with distilled water before being titrated using 0.05 M HCl with phenolphthalein as an indicator. The amount of substituted COOH (n_{COOH}) was calculated using Equation 9, with V_b and V are titrant volume of blank sample and glucomannan sample, respectively. Meanwhile, the DS was calculated using Equation 10.

$$n_{COOH} = (V_b - V) \cdot C_{HCl} \cdot 4 \quad (9)$$

$$DS = \frac{162 \times n_{COOH}}{m_{ds} - 58 \times n_{COOH}} \quad (10)$$

2. 3. 4. Swelling Swelling determination followed the method of Wardhani et al. (12). The glucomannan sample (0.1 g) was dispersed in distilled water (10 ml) at 60°C for 30 min prior to centrifugation at 4000 rpm for 20 min. The pellet was separated by filtration and dried. The weights of wet and dried pellets were recorded for swelling determination using Equation 11.

$$Swelling (\%) = \frac{weight\ of\ wet\ pellet}{weight\ of\ dried\ pellet} \times 100\% \quad (11)$$

2. 3. 5. Functional Groups and Morphology The functional groups of the sample were observed from their

infrared spectra which were obtained using Perkin Elmer Spotlight 200 FTIR (PerkinElmer Inc., Waltham, MA, USA) at the range of 4,000–400 cm^{-1} wavenumber. Meanwhile, the morphology of the samples was captured using an FEI Inspect S50 Scanning Electron Microscope (ThermoFisher Scientific, Waltham, Massachusetts, United States) at 5 kV after the gold-coating process.

2. 3. 6. Emulsion Stability and Optical Microscopy

Ten milliliters of glucomannan emulsion was prepared by homogenizing 0.1 ml of fish oil and the glucomannan solution (1%) using a homogenizer (IKA RW homogenizer, Staufenim Breisgau, Germany) at 12000 rpm for 10 min in an ambient condition. The emulsion stability was determined by observing the height of the cream-serum separation (28). Meanwhile, the optical micrograph of the fresh emulsion was captured using a digital camera connected to a binocular microscope (Sinher XSZ-107BN, Ningbo Beilun Fangyuan Photoelectric Ltd., Zhejiang, China) at 40× objective lens magnification.

2. 4. Statistical Analysis

The obtained data (except the Mw and instrumental data) were performed in triplicate. A one-way analysis of variance (ANOVA) was used for group comparison using Ms. Excel 2019. Data significances were set at $p < 0.05$. The graphs were generated using Sigmaplot (Systat Software Inc., Chicago, IL, USA).

TABLE 1. Viscosity-average molecular weight (Mw) of native and modified glucomannan

Glucomannan treatments	US time (min)	Mw (g/mol)	Decrease of Mw (%)
Native	-	901,175	-
	15	145,711	83.83
US ^a	30	144,183	84.00
	45	142,821	84.15
(US+D) ^b	15	106,586	88.17
	30	93,853	89.58
	45	89,590	90.05
	15	123,499	86.29
(US+CM) ^c	30	119,779	86.70
	45	113,710	87.38
	15	36,247	95.98
	30	20,730	97.70
(US+D+CM) ^d	30	20,730	97.70
	45	10,620	98.82

Note: a=ultrasonication,

b=ultrasonication+deacetylation, subsequently

c=ultrasonication+carboxymethylation, subsequently

d=ultrasonication+deacetylation+carboxymethylation, subsequently

3. RESULTS AND DISCUSSIONS

In this work, the amphiphilic glucomannan was prepared in two steps i.e., deacetylation and carboxymethylation. Prior to these treatments, glucomannan was sonicated to obtain various molecular weights. The effects of the molecular weight on the amphiphilic glucomannan properties after the modifications were studied.

3.1. Glucomannan Degradation Effectiveness of ultrasonication on degrading glucomannan was represented by the decrease of viscosity-average Mw as shown in Table 1. Sonication for 45 min reduced viscosity-average Mw of glucomannan up to 84%. During sonication, the ultrasonic wave formed microbubbles surrounding the glucomannan which then exploded and produced cavitation effect on the glucomannan chain (20). Moreover, the waves also assisted the oscillation of polymer particles and caused more particle collisions (29). Both of those phenomena facilitated molecular degradation and resulted shorter chain of glucomannan, thus decreasing its viscosity-average Mw significantly, as also found by Wardhani et al. (30).

This ultrasonicated glucomannan was subsequently treated with deacetylation, carboxymethylation, or both combined processes. Deacetylation after sonication further reduced the viscosity-average Mw. Deacetylation lowered the viscosity-average Mw as the alkali group that replaced the acetyls of glucomannan had lower Mw (31). Hence, combination ultrasonication and deacetylation decreased the viscosity-average Mw of glucomannan further. Lower Mw of glucomannan after carboxymethylation process was also found by Wang et al. (25). However, the carboxymethyl group has higher Mw than that of the hydroxyl. Hence, the Mw of the carboxymethylation glucomannan was higher compared to that of the deacetylation.

Meanwhile, the viscosity-average Mw of the glucomannan reduced significantly by over 98% when deacetylation and carboxymethylation were applied serially. Replacement of the acetyl group during deacetylation removed steric hindrance of glucomannan to interact with other molecules. This suggested that the deacetylation facilitated the glucomannan to react with the sodium monochloroacetate, in the subsequent step. Moreover, the carboxymethylation produced alkali in the solution as the effect of carboxymethyl attachment, which caused further degradation of glucomannan chain (32).

Therefore, the highest decrease of viscosity-average Mw of glucomannan was found in combination treatment of ultrasonication, deacetylation, and carboxymethylation. Although each deacetylation or carboxymethylation gave further decrease of viscosity-average Mw after ultrasonication, however, the

properties of both glucomannan modification could be different. Hence, the properties of the glucomannan should be considered while choosing the suitable degradation method.

3.2. Degree of Deacetylation Deacetylation is conducted to substitute the acetyl group with the hydroxyl group which are more active in the carboxymethylation process (33). High Mw hindered the substitution reaction during deacetylation and led to have lower DD (Figure 1). Shorter the glucomannan chain improved glucomannan solubility which facilitated the deacetylation reaction as ionization was easier to occur (31). Moreover, lower molecular weight of glucomannan formed lower viscosity of glucomannan solution which eased the molecule mobility and collision (34). Hence, a lower molecular weight of glucomannan is preferable to increase DD.

3.3. Degree of Substitution Ultrasonication and deacetylation, as preliminary treatments prior to the glucomannan carboxymethylation, improved the level of DS (Figure 1). Ultrasonication had a role in lowering glucomannan Mw (Table 1). Xiao et al. (10) and Arifan and Primartu (35) applied deacetylation prior to carboxymethylation to obtain the effective substitution. As mentioned in the earlier section, lowering molecular weight increased the glucomannan solubility and decreased its viscosity, thus leading to ease the glucomannan reaction. High Mw tended to have rigid structures which hindered the carboxymethylation agent from interacting and substituting the hydroxyl groups (36). Deacetylation not only removed the acetyl content of glucomannan but also provided more hydroxyl groups for carboxymethylation substitutions (37). Carboxymethyl groups of sodium monochloroacetate reacted with alkoxides of glucomannan produced after deacetylation/alkalization process (16). Therefore, the

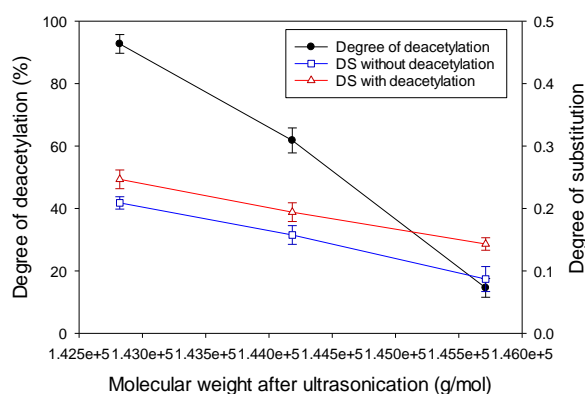


Figure 1. The effect of viscosity-average molecular weight of glucomannan on degree of deacetylation (DD) and degree of substitution (DS)

deacetylation promoted carboxymethylation and increased the DS. Lower Mw due to sonication had indirect effect of increasing degree of substitution through intensifying deacetylation process. Hence, to maximise DS, it is suggested to decrease Mw of polysaccharides and conduct deacetylation process prior to carboxymethylation modification.

3. 4. Swelling Swelling determination represents the ability of glucomannan molecules to expand in aqueous solution. Other than the structural properties, swelling degree of material is also influenced by its charged ions and solvent type (38). Figure 2 shows that ultrasonication improve the swelling of glucomannan. Wang et al. (39) reported that ultrasonication destroyed its crystalline structure which reduced the water-binding ability. Wu et al. (40) found that ultrasonic cavitation also lowered the gel-forming ability of glucomannan.

Deacetylation and carboxymethylation caused further decrease of swelling power. Li et al. (41) explained that water penetration could cause glucomannan swelling through three steps: (i) loosening the lamellar structure of glucomannan, (ii) breaking the intermolecular forces between the lamellar, and (iii) decomposition of amorphous region and severing the crystalline region. Those steps increased the area for water to penetrate. Deacetylation removed the steric hindrance among the glucomannan molecules. This condition allowed glucomannan to interact and reduced the number of water sorption sites (42) resulted lower swelling ability. Carboxymethylation reinforced the glucomannan structure by generating negative charge on the carboxymethylated glucomannan. This negative charged allowed it to interact with proton-hydroxyl group of other glucomannan molecules (36). Decrease of water absorption after glucomannan carboxymethylation was also reported by Xiao et al. (10). Among all glucomannan

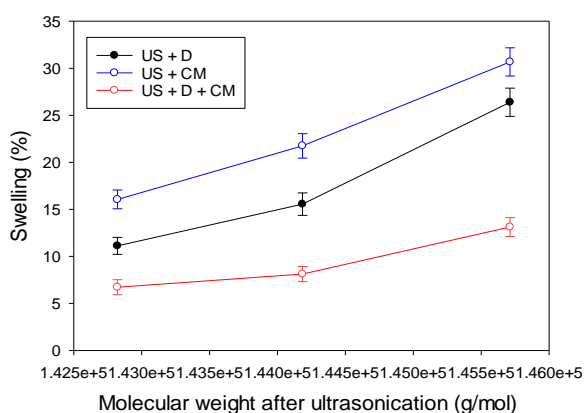


Figure 2. Effect of ultrasonication (US), deacetylation (D), and carboxymethylation (CM) on the swelling of modified glucomannan

treatments in this work, the combination of ultrasonication-deacetylation-carboxymethylation had the most significant effect on decreasing the swelling, hence, this series of reactions gave synergetic work to improve the carboxymethylation reaction and reduced the swelling power.

3. 5. Morphology The surface morphology of the modified glucomannans is shown in Figure 3. Ultrasonicated glucomannan has a rough surface and irregular form. After deacetylation, more wrinkles are observed with deeper cleavages. Different surface morphology is shown by carboxymethylated glucomannan, which has smaller wrinkles. The surface morphology changes after deacetylation and carboxymethylation process were also found by Liu et al. (43) and Xiao et al. (10). The SEM images indicated that chemical modification, i.e. deacetylation and carboxymethylation, also attacked the physical structure of glucomannan.

3. 6. Functional Groups Generally, the modifications applied to the glucomannan did not change the forms of functional groups but their transmittance intensities (Figure 4). Native glucomannan shows its characteristic peaks at $\sim 3325\text{ cm}^{-1}$ (O-H) (44), $\sim 2825\text{ cm}^{-1}$ (C-H), $\sim 1750\text{ cm}^{-1}$ (C=O of the acetyl), $\sim 1250\text{ cm}^{-1}$ (C-O), and $\sim 1000\text{ cm}^{-1}$ (C=O-C). Meanwhile, the mannose group of glucomannan was identified at $\sim 800\text{ cm}^{-1}$. Ultrasonication and deacetylation increased the peak of O-H group. Ultrasonication increased the hydroxyl content of glucomannan as the effect of its chain-breaking (30), while the increase after deacetylation indicated that the hydroxyl group attachment occurred during the reaction. Jin et al. (45) also found an increasing O-H group intensity after deacetylation, which also showed an increase in intermolecular hydrogen bonds (46).

Interestingly, almost all peaks of the sonicated-carboxymethylated sample showed the lowest intensity of transmittance except on $\sim 1400\text{ cm}^{-1}$ which is related to $-\text{CH}_2-$ group. Deacetylation removed C=O of acetyl from the sonicated sample at $\sim 1750\text{ cm}^{-1}$ due to alkali treatment. However, carboxymethylation reintroduced this group back to the sample, resulting in lower transmittance. The peaks of band at ~ 1250 and $\sim 1017\text{ cm}^{-1}$, which indicated the group of C-O and C=O-C, were intensified after carboxymethylation process. Stronger after carboxymethylation was also found by Wang et al. (25) and Chen et al. (47). Moreover, number of intramolecular hydrogen binding were found to increase after the carboxymethylation, as described by reducing the transmittance at $\sim 1640\text{ cm}^{-1}$ (25). Therefore, the IR spectra showed that lower Mw promoted the carboxymethylation process, even without glucomannan deacetylation.

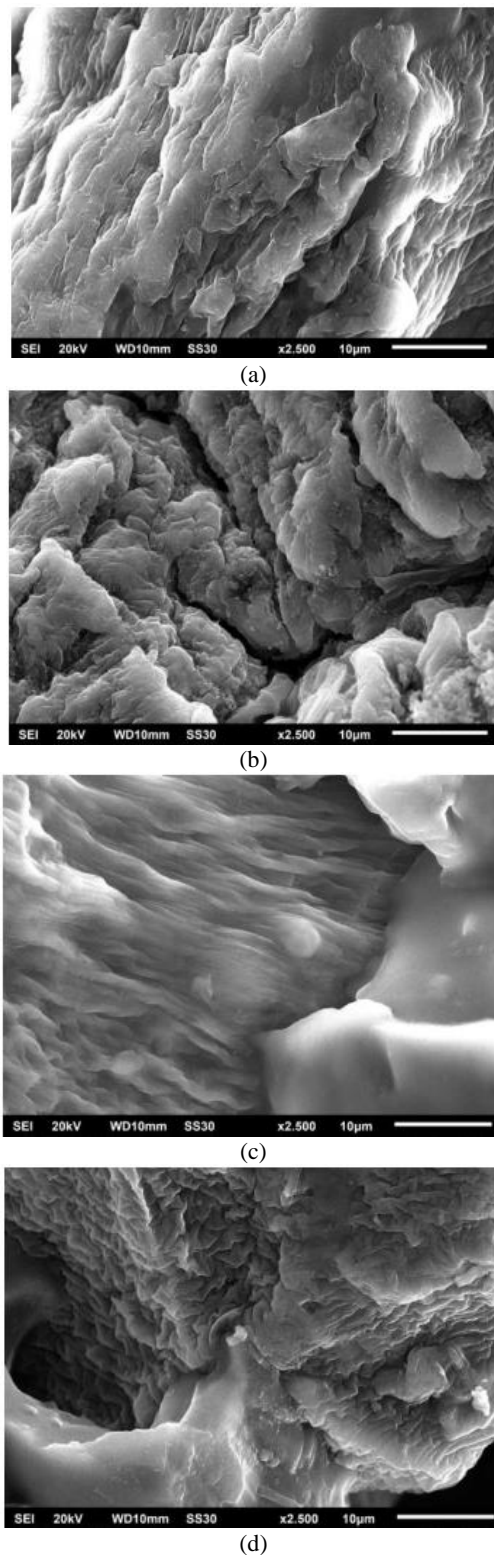


Figure 3. Surface morphology at 2,500 \times magnification of glucomannan after (a) ultrasonication, (b) ultrasonication-deacetylation, (c) ultrasonication-carboxymethylation, (d) ultrasonication-deacetylation-carboxymethylation

3. 7. Emulsion Stability Performance of native and amphiphilic glucomannan on stabilizing o/w emulsion was determined by observing development of phase separation of the emulsion during 300 h storage at room temperature. The highest DS sample (DS=0.25) was selected to represent the amphiphilic glucomannan. The emulsion of the native glucomannan was separated after 180 h of storage (Figure 5). Meanwhile, the amphiphilic emulsion started to separate after 240 h. By the end of storage duration (300 h), the phase separation of the native and the amphiphilic emulsions was 42.88% and 5.15%, respectively.

The emulsion stability was also supported by the micrograph of glucomannan-fish oil droplets (Figure 6). After 300 h, more smaller droplets were observed in the amphiphilic glucomannan emulsion compared to those of

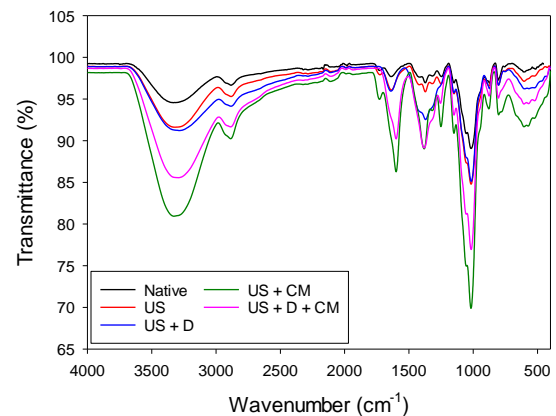


Figure 4. IR spectra of native glucomannan and modified glucomannan using ultrasonication (US), deacetylation (D), and carboxymethylation (CM)

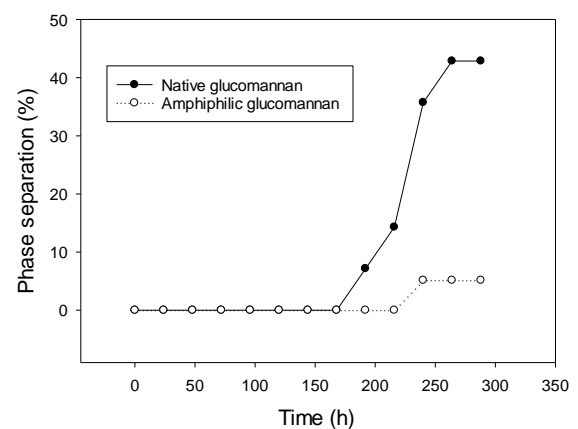


Figure 5. Phase separation of fish oil emulsion stabilized using native and amphiphilic glucomannan

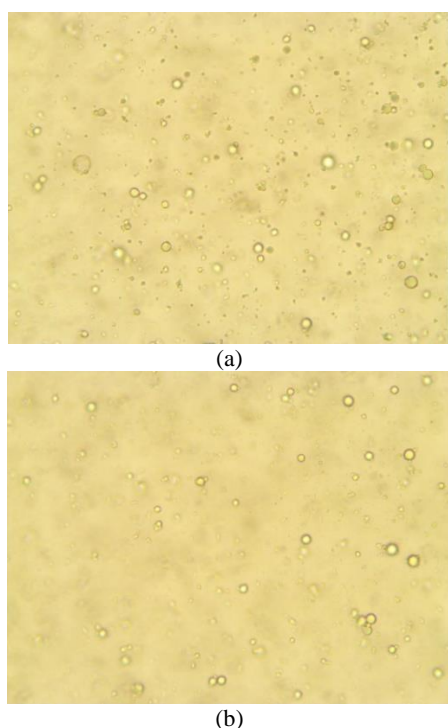


Figure 6. Optical micrograph of fish oil emulsion right after homogenization stabilized using (a) native and (b) amphiphilic glucomannan

the native one. Droplet size of the emulsion was known as one of the emulsion stability indicators as smaller droplets slowed down the rate of droplet coalescence (48). Hence, the amphiphilic glucomannan could maintain the oil droplets in glucomannan solution and stabilize the emulsion. Other than droplet size, o/w emulsion stability was also influenced by viscosity, pH, ionic content, mixing variables, and interaction between dispersed and continuous phases (49).

As shown in Figure 4, the amphiphilic glucomannan has lower transmittance of carboxylate and methylene groups which have a hydrophobic property (50, 51). These hydrophobic groups are allowed to bond with fish oil during dissolving the oil in water. Cai et al. (52) also found a stable emulsion when used carboxymethyl starch in pH variation. This result suggested that serial treatment using ultrasonication, deacetylation, and carboxymethylation succeeded in improving the hydrophobic properties of glucomannan, thus, enhance its emulsifying properties. However, apart of the hydrophobic modification of glucomannan and droplet size, other factors should be examined in the future to precise the influencing factors on stabilized emulsion.

4. CONCLUSION

Natural emulsifier produced by native natural ingredient is lacked for its performance on stabilizing o/w emulsion.

Decreasing the length of hydrophilic chain is known to increase the emulsifying properties of surfactants. In this study, amphiphilic glucomannan has been produced using carboxymethylation method which is supported by ultrasonication to initially degrade the glucomannan to obtain various Mw. Ultrasonication, deacetylation, and carboxymethylation performed synergetic work on decreasing the Mw of glucomannan and swelling power, while enhancing the DD of deacetylation and also the value of DS of carboxymethylation. The modifications changed the morphology and intensities of the transmittance peaks as shown in IR spectra of glucomannan. The amphiphilic glucomannan was successfully stabilized fish oil emulsion with ~5% phase separation after 300 h. This reduced separation exhibited the preferential stabilizing properties of amphiphilic glucomannan which is potential for food application. This study increased the probability to use natural surfactant for emulsion stabilization and encapsulation. The efficacy of the amphiphilic glucomannan to stabilize the emulsion of real foods and its impact on the food properties become a target for future study.

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6. REFERENCES

1. Srzednicki G, Borompichaichartkul C. Konjac glucomannan: Production, processing, and functional applications: CRC Press; 2020.
2. Koroskenyi B, McCarthy SP. Synthesis of acetylated konjac glucomannan and effect of degree of acetylation on water absorbency. *Biomacromolecules*. 2001;2(3):824-6. <https://doi.org/10.1021/bm010014c>
3. Wu K, Zhu Q, Qian H, Xiao M, Corke H, Nishinari K, et al. Controllable hydrophilicity-hydrophobicity and related properties of konjac glucomannan and ethyl cellulose composite films. *Food Hydrocolloids*. 2018;79:301-9. <https://doi.org/10.1016/j.foodhyd.2017.12.034>
4. Dan N. Core-shell drug carriers: liposomes, polymersomes, and niosomes. *Nanostructures for drug delivery*: Elsevier; 2017. p. 63-105.
5. Cunha AG, Gandini A. Turning polysaccharides into hydrophobic materials: a critical review. Part 2. Hemicelluloses, chitin/chitosan, starch, pectin and alginates. *Cellulose*. 2010;17:1045-65. <https://doi.org/10.1007/s10570-010-9435-5>
6. Nworie FS, Mgbemena N, Ike-Amadi A, Ebunoha J. Functionalized Biochars for Enhanced Removal of Heavy Metals from Aqueous Solutions: Mechanism and Future Industrial Prospects. *Journal of Human, Earth, and Future*. 2022;3(3):377-95. <https://doi.org/10.28991/HEF-2022-03-03-09>

7. Zhang Y-q, Xie B-j, Gan X. Advance in the applications of konjac glucomannan and its derivatives. *Carbohydrate polymers*. 2005;60(1):27-31. <https://doi.org/10.1016/j.carbpol.2004.11.003>
8. Cova A, Sandoval AJ, Balsamo V, Müller AJ. The effect of hydrophobic modifications on the adsorption isotherms of cassava starch. *Carbohydrate Polymers*. 2010;81(3):660-7. <https://doi.org/10.1016/j.carbpol.2010.03.028>
9. Jin Y, Zhang H, Yin Y, Nishinari K. Comparison of curdlan and its carboxymethylated derivative by means of Rheology, DSC, and AFM. *Carbohydrate Research*. 2006;341(1):90-9. <https://doi.org/10.1016/j.carres.2005.11.003>
10. Xiao M, Dai S, Wang L, Ni X, Yan W, Fang Y, et al. Carboxymethyl modification of konjac glucomannan affects water binding properties. *Carbohydrate Polymers*. 2015;130:1-8. <https://doi.org/10.1016/j.carbpol.2015.05.001>
11. Nematov D, Kholmurodov K, Husenzoda M, Lyubchik A, Burhonzoda A. Molecular Adsorption of H₂O on TiO₂ and TiO₂: Y Surfaces. *Journal of Human, Earth, and Future*. 2022;3(2):213-22. <https://doi.org/10.28991/HEF-2022-03-02-07>
12. Wardhani DH, Nugroho F, Aryanti N, Prasetyaningrum A. Simultaneous effect of temperature and time of deacetylation on physicochemical properties of glucomannan. *ASEAN Journal of Chemical Engineering*. 2018;18(1):1-8.
13. Qiao D, Lu J, Shi W, Li H, Zhang L, Jiang F, et al. Deacetylation enhances the properties of konjac glucomannan/agar composites. *Carbohydrate Polymers*. 2022;276:118776. <https://doi.org/10.1016/j.carbpol.2021.118776>
14. Wardhani DH, Ulya HN, Nugroho F, Sasongko SB, Hadiyanto A, Nugroho A. Vitamin C encapsulation by a gelation method using deacetylated glucomannan as a matrix. *Journal of King Saud University-Science*. 2020;32(7):2924-30. <https://doi.org/10.1016/j.jksus.2020.07.010>
15. Du X, Li J, Chen J, Li B. Effect of degree of deacetylation on physicochemical and gelation properties of konjac glucomannan. *Food Research International*. 2012;46(1):270-8. <https://doi.org/10.1016/j.foodres.2011.12.015>
16. Aprilia V, Murdiati A, Hastuti P, Harmayani E. Carboxymethylation of glucomannan from porang tuber (*Amorphophallus oncophyllus*) and the physicochemical properties of the product. *Pakistan Journal of Nutrition*. 2017;16(11):835-42. <https://doi.org/10.3923/pjn.2017.835.842>
17. Day RA, Estabrook DA, Wu C, Chapman JO, Togle AJ, Sletten EM. Systematic study of perfluorocarbon nanoemulsions stabilized by polymer amphiphiles. *ACS applied materials & interfaces*. 2020;12(35):38887-98. <https://doi.org/10.1021/acsami.0c07206>
18. Li B, Xie B. Synthesis and characterization of konjac glucomannan/poly (vinyl alcohol) interpenetrating polymer networks. *Journal of applied polymer science*. 2004;93(6):2775-80. <https://doi.org/10.1002/app.20769>
19. Li J, Li B, Geng P, Song A-X, Wu J-Y. Ultrasonic degradation kinetics and rheological profiles of a food polysaccharide (konjac glucomannan) in water. *Food Hydrocolloids*. 2017;70:14-9. <https://doi.org/10.1016/j.foodhyd.2017.03.022>
20. Wardhani DH, Aryanti N, Aziz A, Firdhaus RA, Ulya HN. Ultrasonic degradation of alginate: A matrix for iron encapsulation using gelation. *Food Bioscience*. 2021;41:100803. <https://doi.org/10.1016/j.fbio.2020.100803>
21. Feng L, Cao Y, Xu D, Wang S, Zhang J. Molecular weight distribution, rheological property and structural changes of sodium alginate induced by ultrasound. *Ultrasonics sonochemistry*. 2017;34:609-15. <https://doi.org/10.1016/j.ultsonch.2016.06.038>
22. Low S, Tan M, Chin N, Tan K. Comparative Studies on Ultrasound Pre-treated Peanut Husk Powder and Ultrasound Simultaneous Process on Heavy Metal Adsorption. *International Journal of Engineering*. 2018;31(8):1334-40. <https://doi.org/10.5829/ije.2018.31.08b.23>
23. Wu Z, Song X. Carboxymethylation of γ -irradiated starch. *Journal of applied polymer science*. 2006;101(4):2210-5. <https://doi.org/10.1002/app.23479>
24. Wardhani DH, Cahyono H, Ulya HN, Kumoro AC, Anam K, Vázquez JA. Spray-dryer feed preparation: Enzymatic degradation of glucomannan for iron nanoencapsulation. 2022. <https://doi.org/10.3934/agrfood.2022042>
25. Wang M, He W, Wang S, Song X. Carboxymethylated glucomannan as paper strengthening agent. *Carbohydrate polymers*. 2015;125:334-9. <https://doi.org/10.1016/j.carbpol.2015.02.060>
26. Ratcliffe I, Williams PA, Viebke C, Meadows J. Physicochemical characterization of konjac glucomannan. *Biomacromolecules*. 2005;6(4):1977-86. <https://doi.org/10.1021/bm0492226>
27. Distantina S, Kaavessina M, Fadilah F, Putrie AS, Novianti I, editors. Carboxymethyl konjac glucomannan from konjac flour: The effect of media and temperature on carboxymethylation rate. *AIP Conference Proceedings*; 2018: AIP Publishing.
28. Huang X, Kakuda Y, Cui W. Hydrocolloids in emulsions: particle size distribution and interfacial activity. *Food hydrocolloids*. 2001;15(4-6):533-42. [https://doi.org/10.1016/S0268-005X\(01\)00091-1](https://doi.org/10.1016/S0268-005X(01)00091-1)
29. Li J, Cai J, Fan L. Effect of sonolysis on kinetics and physicochemical properties of treated chitosan. *Journal of Applied Polymer Science*. 2008;109(4):2417-25. <https://doi.org/10.1002/app.28339>
30. Wardhani DH, Ulya HN, Maulana I, Salsabila S, Kumoro AC, Vázquez JA. Analyzing the characteristics of degraded glucomannan of *Amorphophallus oncophyllus* using hydrogen peroxide and ultrasonication. 2023. <https://doi.org/10.3934/agrfood.2023031>
31. Ulya HN, Wardhani DH, Aryanti N, Pangestuti DR, editors. Deacetylation of glucomannan of *Amorphophallus oncophyllus* using NaOH and its properties as iron excipient by gelation in ethanol. *AIP Conference Proceedings*; 2019: AIP Publishing.
32. El-Sheikh MA. Carboxymethylation of maize starch at mild conditions. *Carbohydrate Polymers*. 2010;79(4):875-81. <https://doi.org/10.1016/j.carbpol.2009.10.013>
33. Fadilah F, Distantina S, Kaavessina M, Wijayanti ST, Andayani R, editors. Study on the carboxymethylation of glucomannan from porang. *AIP Conference Proceedings*; 2018: AIP Publishing.
34. Guo L, Yokoyama W, Chen L, Liu F, Chen M, Zhong F. Characterization and physicochemical properties analysis of konjac glucomannan: Implications for structure-properties relationships. *Food Hydrocolloids*. 2021;120:106818. <https://doi.org/10.1016/j.foodhyd.2021.106818>
35. Arifan F, Primartu S. Physical and chemical characteristics of carboxymethyl cellulose from Kepok banana (*Musa paradisiaca* formatypica) and corncobs (*Zea mays*) modified alkalization-carboxymethylation process in ice cream products. *Food Research*. 2023;7(2):31-6. [https://doi.org/10.26656/fr.2017.7\(2\).355](https://doi.org/10.26656/fr.2017.7(2).355)
36. Wu J, Zhong F, Li Y, Shoemaker C, Xia W. Preparation and characterization of pullulan-chitosan and pullulan-carboxymethyl chitosan blended films. *Food Hydrocolloids*. 2013;30(1):82-91. <https://doi.org/10.1016/j.foodhyd.2012.04.002>
37. Li J, Ye T, Wu X, Chen J, Wang S, Lin L, et al. Preparation and characterization of heterogeneous deacetylated konjac glucomannan. *Food Hydrocolloids*. 2014;40:9-15. <https://doi.org/10.1016/j.foodhyd.2014.02.001>

38. Gani BA, Asmah N, Soraya C, Syafriza D, Rezeki S, Nazar M, et al. Characteristics and Antibacterial Properties of Film Membrane of Chitosan-Resveratrol for Wound Dressing. *Emerging Science Journal*. 2023;7(3):821-42. <https://doi.org/10.28991/ESJ-2023-07-03-012>
39. Wang M, Wu Y, Liu Y, Ouyang J. Effect of ultrasonic and microwave dual-treatment on the physicochemical properties of chestnut starch. *Polymers*. 2020;12(8):1718. <https://doi.org/10.3390/POLYM12081718>
40. Wu H, Tong L, Wang Y, Yan H, Sun Z. Bibliometric analysis of global research trends on ultrasound microbubble: a quickly developing field. *Frontiers in Pharmacology*. 2021;12:646626. <https://doi.org/10.3389/fphar.2021.646626>
41. Li L, Ruan H, Ma L-I, Wang W, Zhou P, He G-q. Study on swelling model and thermodynamic structure of native konjac glucomannan. *Journal of Zhejiang University SCIENCE B*. 2009;10:273-9. <https://doi.org/10.1631/jzus.B0820221>
42. Huang Y-C, Chu H-W, Huang C-C, Wu W-C, Tsai J-S. Alkali-treated konjac glucomannan film as a novel wound dressing. *Carbohydrate Polymers*. 2015;117:778-87. <https://doi.org/10.1016/j.carbpol.2014.10.047>
43. Liu X, Gan J, Nirasawa S, Tatsumi E, Yin L, Cheng Y. Effects of sodium carbonate and potassium carbonate on colloidal properties and molecular characteristics of konjac glucomannan hydrogels. *International journal of biological macromolecules*. 2018;117:863-9. <https://doi.org/10.1016/j.ijbiomac.2018.05.176>
44. Yusmaniar Y, Julio E, Rahman A, Yudanto SD, Susetyo FB. Synthesis of Polyvinyl Alcohol-Chitosan Composite Film using Nanocellulose from Coconut Fibers (*Cocos nucifera*). *International Journal of Engineering*. 2023;36(11):1993-2003. <https://doi.org/10.5829/ije.2023.36.11b.05>
45. Jin W, Song R, Xu W, Wang Y, Li J, Shah BR, et al. Analysis of deacetylated konjac glucomannan and xanthan gum phase separation by film forming. *Food Hydrocolloids*. 2015;48:320-6. <https://doi.org/10.1016/j.foodhyd.2015.02.007>
46. Solo-de-Zaldívar B, Tovar C, Borderías A, Herranz B. Effect of deacetylation on the glucomannan gelation process for making restructured seafood products. *Food Hydrocolloids*. 2014;35:59-68. <https://doi.org/10.1016/j.foodhyd.2013.04.009>
47. Chen L, Du Y, Tian Z, Sun L. Effect of the degree of deacetylation and the substitution of carboxymethyl chitosan on its aggregation behavior. *Journal of Polymer Science Part B: Polymer Physics*. 2005;43(3):296-305. <https://doi.org/10.1021/bm010014c>
48. Wardhani DH, Ulya HN, Kumoro AC, Aryanti N. Partial hydrophobic modification of alginate using dodecyl succinic anhydride for fish oil encapsulation. *Acta Scientiarum Polonorum Technologia Alimentaria*. 2022;21(4):369-78. <https://doi.org/10.17306/J.AFS.2022.1077>
49. Maphosa Y, Jideani VA. Factors affecting the stability of emulsions stabilised by biopolymers. *Science and technology behind Nanoemulsions*. 2018;65. <https://doi.org/10.5772/intechopen.75308>
50. Spychaj T, Wilpiszewska K, Zdanowicz M. Medium and high substituted carboxymethyl starch: synthesis, characterization and application. *Starch-Stärke*. 2013;65(1-2):22-33. <https://doi.org/10.1002/star.201200159>
51. Yanli W, Wenyuan G, Xia L. Carboxymethyl Chinese yam starch: synthesis, characterization, and influence of reaction parameters. *Carbohydrate Research*. 2009;344(13):1764-9. <https://doi.org/10.1016/j.carres.2009.06.014>
52. Cai X, Wang Y, Du X, Xing X, Zhu G. Stability of pH-responsive Pickering emulsion stabilized by carboxymethyl starch/xanthan gum combinations. *Food Hydrocolloids*. 2020;109:106093. <https://doi.org/10.1016/j.foodhyd.2020.106093>

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Persian Abstract

چکیده

کربوکسی متیلاسیون گلوکومانان برای افزایش خواص آبریز آن و گسترش تعامل آن با ترکیبات آبریز انجام می شود. با این حال، گلوکومانان دارای وزن مولکولی بالا و یک زنجیره پلی ساکارید طولانی است که از تغییر خواص آمفیفیلیک آن جلوگیری می کند. این مطالعه با هدف بررسی اثر وزن مولکولی، استیل زدایی و کربوکسی متیلاسیون بر خواص گلوکومانان انجام شد. عملکرد گلوکومانان اصلاح شده برای تثبیت امولسیون های روغن در آب (o/w) نیز مورد مطالعه قرار گرفت. برای به دست آوردن وزن های مولکولی مختلف، فراصوت به گلوکومانان با فرکانس 40 کیلوهرتز به مدت 15-45 دقیقه اعمال شد. کربنات سدیم (Na_2CO_3) و مونوکلوآستات سدیم به ترتیب به عنوان عوامل استیل زدایی و کربوکسی متیلاسیون استفاده شد. نتایج نشان می دهد که کاهش وزن مولکولی از فرآیند استیل زدایی و کربوکسی متیلاسیون در اتصال گروه های آبریز به زنجیره گلوکومانان پشتیبانی می کند، بنابراین، خواص آبدوستی و درجه تورم گلوکومانان را کاهش می دهد. تغییرات ساختاری و مورفولوژیکی گلوکومانان پس از تغییرات از طریق طیف IR و تصاویر SEM تایید شد. عملکرد عالی گلوکومانان آمفیفیلیک در تثبیت امولسیون o/w مشاهده شد زیرا تنها 5 درصد جداسازی فاز پس از 300 ساعت ذخیره سازی در شرایط محیطی رخ داد. از این رو، سونوگرافی به عنوان یک درمان اولیه مناسب برای تولید گلوکومانان آمفیفیلیک پیشنهاد می شود. a.