

## Modification of hydrophobic properties of konjac glucomannan using octenyl succinic anhydride

<sup>1,2</sup>Widarta, I.W.R., <sup>2,\*</sup>Raharjo, S., <sup>2</sup>Santoso, U. and <sup>2</sup>Supriyadi

<sup>1</sup>Department of Food Technology, Faculty of Agricultural Technology, Udayana University, Bali 80361, Indonesia

<sup>2</sup>Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia

### Article history:

Received: 1 December 2021

Received in revised form: 5 January 2021

Accepted: 21 April 2022

Available Online: 29 September 2023

### Keywords:

Emulsifier,  
Konjac glucomannan,  
Emulsion,  
Sodium carbonate,  
Octenyl succinic anhydride

### DOI:

[https://doi.org/10.26656/fr.2017.7\(5\).910](https://doi.org/10.26656/fr.2017.7(5).910)

### Abstract

Konjac glucomannan (KGM) is a water-soluble polysaccharide that has the potential to be used as a natural emulsifier because of its high viscosity. This study aimed to modify the KGM structure in order to obtain a KGM which has amphiphilic properties. Modifications were carried out in the microwave using 1-3% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and 2-4% octenyl succinate anhydride (OSA). Parameters observed included emulsion capacity, emulsion stability, degree of substitution with High-Performance Liquid Chromatography (HPLC), viscosity, hydrophobicity, and Fourier transform infrared Spectroscopy (FT-IR). The results showed that the interaction between the concentration of  $\text{Na}_2\text{CO}_3$  and OSA had a significant effect on the capacity and stability of the octenyl succinic anhydride-modified konjac glucomannan (KGOS) emulsion. The highest emulsion capacity and stability were obtained using 2%  $\text{Na}_2\text{CO}_3$  and 4% OSA. OSA esterification of KGM was shown by increasing the degree of substitution, viscosity, hydrophobicity, and FT-IR analysis. Thus, KGOS can be used as a natural emulsifier with good amphiphilic properties.

## 1. Introduction

An emulsion is a heterogeneous system consisting of at least 2 phases. Emulsifiers are added to decrease the interfacial surface tension and increase the stability of the emulsion by increasing the repulsion forces between the droplets. Emulsifiers are widely applied in the food industry, cosmetics, pharmacy and medicine, biotechnology, agrochemicals, petroleum, fabric, leather or metal processing, pulp and paper industry, paints and lacquers, detergence, lubrication (Bendjaballah *et al.*, 2010; Zhang *et al.*, 2017). Currently, synthetic emulsifiers are more dominantly used because they are practical in their application, but natural emulsifiers are better used because of their lower toxicity and are highly biodegradable (Sapei *et al.*, 2017). Natural emulsifiers can be obtained by modifying the chemical structure of polysaccharides (Dapčević Hadnadev *et al.*, 2014; Zhang *et al.*, 2021). Zhang *et al.* (2017) reported that polysaccharides also have an additional role as a stabilizer to increase the viscosity of the continuous phase can slow the movement of the droplets, prevent phase separation and improve the long-term physical stability of emulsions.

Polysaccharides commonly used as surfactants are

hydroxypropyl methylcellulose (HPMC), but their application needs stabilizers such as sodium alginate, Arabic gum, flaxseed gum, guar gum, locust bean gum and xanthan gum were chosen to stabilize the emulsion with HPMC as the main emulsifier (Meng *et al.*, 2018a). Octenyl succinic anhydride-modified starch (OSA-starch) also could act as an effective emulsifier due to containing both hydrophilic and hydrophobic groups. OSA-starch is obtained by the esterification reaction between starch and anhydrous octenyl succinic acid under alkaline conditions (Dapčević Hadnadev *et al.*, 2014), however, OSA-starch has low emulsion capacity and stability (Meng *et al.*, 2014). Another type of polysaccharide that can be used as an emulsifier is KGM isolated from konjac tubers (*Amorphophallus konjac*) (Zhong *et al.*, 2018).

KGM is a dietary fibre with a long history in food and traditional Chinese medicine. The KGM main chain is polymerized by D-mannose and D-glucose with an  $\alpha$ -1,4-pyranoside bond and a small number of acetyl groups at the C-6 position of the side chain (Yang *et al.*, 2017). KGM has already been approved by many countries or organizations as generally regarded as safe (GRAS) in foods. KGM has a high molecular weight

\*Corresponding author.

Email: [sraharjo@ugm.ac.id](mailto:sraharjo@ugm.ac.id)

range of (Mw)  $10^5$ - $10^6$  (Zhong *et al.*, 2018). This hydrophilic and biodegradable compound is known as one of the highest viscosity copolymers. It has an extraordinary water-binding capacity and is able to absorb over 100 times its dry weight (Wardhani *et al.*, 2018). Structure modification is needed to obtain its amphiphilic KGM properties. KGM modification is carried out in alkaline processing so that the amount of alkali used affects the success of the modification process. Li *et al.* (2014) reported that  $\text{Na}_2\text{CO}_3$  can be used as an alkalinizing agent which plays a role in the deacetylation of the acetyl group in KGM while anhydrous octenyl succinate is used to give hydrophobic properties to KGM. The degree of OSA substitution is strongly influenced by the amount of OSA added. The deacetylation and esterification reactions of OSA in KGM occur simultaneously using the microwave method (Meng *et al.*, 2014). This research aimed to obtain the appropriate concentration of  $\text{Na}_2\text{CO}_3$  and OSA so as to produce OSA-modified KGM with higher capacity and emulsion stability.

## 2. Materials and methods

### 2.1 Materials

KGM with 99.88% purity was purchased from an online marketplace, and was extracted and refined from Konjac root (*Amorphophallus konjac*). The 2-Octen-1-ylsuccinic anhydride (OSA, 99.0% purity), ethanol absolute, and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO) and hydrochloric acid 37% was purchased from Mallinckrodt Baker, Inc. All chemicals were analytical reagents unless otherwise stated.

### 2.2 Preparation of konjac glucomannan

The KGOS was prepared using a microwave method as described previously (Meng *et al.*, 2014). Twenty grams (dry weight) of KGM powder and 1-3%  $\text{Na}_2\text{CO}_3$  (in proportion to KGM, w/w) were added to the reaction vessel. After homogeneous mixing, 80.00 mL of ethanol solution (30%) was added slowly with agitation and 2-4% of OSA (in proportion to KGM, w/w), was added (diluted five times with absolute ethanol) with slow agitation. The mixture was placed in the centre of an Electrolux microwave reactor (Stockholm, Sweden), heated at 800 W for 3 mins, cooled to 25°C, and blended with 40.00 mL of 30% ethanol solution for 5 mins. The pH of the solution was adjusted to 6.50 with HCl solution (1 N). The mixture was centrifuged at 3000 rpm for 10 min and filtered, washing with 30% ethanol five times to remove residues of  $\text{Na}^+$ ,  $\text{Cl}^-$  and other soluble impurities and with absolute ethanol five times to remove OSA

residues. The final solid portion was oven-dried at 50°C for 3 hrs and passed through a 100-mesh nylon sieve.

### 2.3 Viscosity, emulsion capacity and emulsion stability tests

One percent of KGOS and KGM solutions were heated at 60°C for 20 mins and then cooled to room temperature. A viscometer Brookfield (DV2T, US) was used to determine the apparent viscosity of the solution at  $30 \pm 0.5^\circ\text{C}$  at 30 r/mins with spindle #64. The peak viscosity of the solution was defined as the final viscosity.

The emulsion capacity (EC) and emulsion stability (ES) were tested according to Meng *et al.* (2018b) with some modifications: First, 0.1 g of a KGOS sample was placed in a 100 mL beaker, 40 g of distilled water was added, and the mixture was stirred at 60°C for 20 mins. After cooling to room temperature, 10.00 g of corn germ oil was added gradually and homogenized using an Ultra-turrax Homogenizer (T50 Basic, IKA-Werke, Germany) at 6000 rpm for 1 min. Then, the emulsions were transferred to 50 mL tubes and centrifuged (DM0636 Multi-purpose centrifuge, DLAB, US) at 3000 rpm for 10 min. The EC was calculated as follows:

$$\text{EC} = (\text{Height of emulsion layer} / \text{total height}) \times 100\%$$

Each emulsion was stored at room temperature. The ES of the samples was the EC after 30 days, calculated as follows:

$$\text{ES} = (\text{Height of remaining emulsion layer} / \text{total height}) \times 100\%$$

### 2.4 Degree of substitution

The degree of substitution (DS) of PGOS was measured by high-performance liquid chromatography (HPLC) using methods developed previously (Zhong *et al.*, 2018). HPLC analysis was performed by an LC-20A HPLC (Shimadzu, Japan) equipped with a Thermo BDS C18 column (Shim-Pack GIST, 5  $\mu\text{m}$  C18; 4.6  $\times$  150 mm, Shimadzu, Japan) and a UV detector (Shimadzu, Japan) operated at 200 nm. A mixture of acetonitrile and water with 0.1% TFA (55:45, v/v) was used as the mobile phase. The flow rate was 1.0 mL/min.

### 2.5 Standard curves for octenyl succinate anhydride

OSA reagent (0.1052 g) was added to a 25 mL volumetric flask and diluted to the desired volume (25 mL) with acetonitrile. Approximately 3 mL of OSA solution was pipetted into 25 mL beakers, followed by 5 mL 1 M NaOH ethanol solution (30%), respectively. Samples were covered with plastic wrap and

magnetically stirred at 400 r/min for 12 hrs. Then, 1.5 mL 4 M HCl ethanol solution (30%) was pipetted into beakers and the mixture was transferred into 10 mL volumetric flasks after cooling to room temperature and filled with acetonitrile to a total of 10 mL and then pipetted 0.5, 1, 1.5, 2, or 2.5 mL this solution into 5 mL volumetric flask and dilute to the desired volume (5 mL) with acetonitrile.

### 2.5.1 Determination of free and bound octenyl succinate anhydride

To determine the free OSA content of PGOS, 0.1000 g PGOS (dry weight) was added to a 50 mL beaker glass and extracted with 5 mL of methanol with magnetic stirring for 1 hrs. The mixture was centrifuged, and 1 mL of supernatant was collected into a beaker and mixed with 5 mL 1 M NaOH ethanol solution (30%) and magnetic stirring at 400 r/min for 12 hrs. Next, 1.5 mL 4 M HCl ethanol solution (30%) was pipetted into a beaker glass and cooled to room temperature; residues were removed using a 0.45  $\mu\text{m}$  membrane filter, and 3 mL was pipetted into a 10 mL volumetric flask and filled with acetonitrile to a total of 10 mL. The OSA content ( $W_{\text{free}}$ , g) was calculated using the total peak area ( $A_{\text{free}}$ ) and the standard curve.

For total OSA content determination, PGOS (0.1000 g dry weight) was weighed into a 50 mL beaker glass, 5 mL 1 M NaOH ethanol solution (30%) was added, and the solution was magnetically stirred at 400 r/min for 12 h. Next, 1.5 mL 4 M HCl ethanol solution (30%) was pipetted into a centrifuge tube and cooled to room temperature. Residues were removed with a 0.45  $\mu\text{m}$  membrane filter, and 3 mL was pipetted into a 10 mL volumetric flask and filled with acetonitrile to a total of 10 mL. The OS in the solution was analyzed by HPLC to yield a total peak area ( $A_{\text{total}}$ ) and corresponding weight ( $W_{\text{total}}$ , g) using a standard curve. The Degree of substitution (DS) was calculated using the following equation:

$$\text{DS} = \frac{\text{OSA total} - \text{OSA free}}{\text{mPGOS}} \times 100 \%$$

Where mPGOS is the dry weight of PGOS

### 2.6 Hydrophobicity

The main parameter of hydrophobicity of a material is the static contact angle, which is defined as the angle formed between a liquid and a solid. The water contact angle is measured by a contact angle meter (DSA25, Krüss Co., Ltd., Germany) equipped with a CCD camera and image analysis software. PGOS (0.25 g) was dissolved in 25 mL of water and then heated at 60°C for 20 mins. The PGOS solution was placed in a plastic mould with a diameter of 8 cm and dried in a cabinet

dryer for 24 hrs to obtain a film. A droplet of distilled water (3.0  $\mu\text{L}$ ) was deposited on the airside surface of the film (2.0 cm  $\times$  2.0 cm) with a precision syringe, and the drop image was recorded by a camera. The contact angle was measured after stabilizing for 45 s (Li *et al.*, 2019).

### 2.7 Fourier transform infrared spectroscopy

The KGM and the KGOS were qualitatively analyzed using an FT-IR spectrometer (Spectrum 100; Thermo Scientific Nicolet iS10, USA) in a wavenumber range of 450–4000  $\text{cm}^{-1}$  (Meng *et al.*, 2018b). The samples were ground and mixed with KBr (1:50), then dried at 105°C for 12 hrs and pressed into tablets for analysis.

### 2.8 Statistical analysis

The analysis was conducted using a completely randomized design. All experiments were carried out in triplicate and the results were represented by means and standard deviations. Analysis of variance (ANOVA) was computed using SPSS 16.0 and Duncan's Multiple Range Test was conducted to examine significant differences among experimental mean values ( $p < 0.05$ ).

## 3. Results and discussion

### 3.1 Influence of $\text{Na}_2\text{CO}_3$ and octenyl succinate anhydride concentration on KGOS emulsion capacity

The correlation between  $\text{Na}_2\text{CO}_3$  and OSA concentration on emulsion capacity is shown in Figure 1. The ANOVA result showed that the interaction between  $\text{Na}_2\text{CO}_3$  and OSA concentrations has a significant effect on KGOS emulsion capacity ( $P < 0.05$ ). The addition of 2%  $\text{Na}_2\text{CO}_3$  and 4% OSA increased the emulsion capacity up to 42.17%. Jian *et al.* (2015) report that acidic and alkaline conditions can hydrolyze acetyl groups in KGM. Increasing the concentration of  $\text{Na}_2\text{CO}_3$

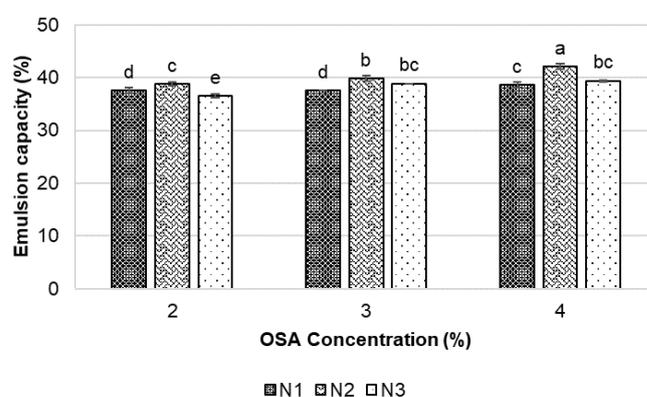


Figure 1. Effect of  $\text{Na}_2\text{CO}_3$  and OSA concentrations on emulsion capacity. N1: 1%  $\text{Na}_2\text{CO}_3$ , N2: 2%  $\text{Na}_2\text{CO}_3$ , N3: 3%  $\text{Na}_2\text{CO}_3$ . Bars with different notations are statistically significantly different ( $p < 0.05$ ).

can increase the degree of deacetylation (Wardhani *et al.*, 2017). However, the higher concentration of Na<sub>2</sub>CO<sub>3</sub> inhibited the esterification reaction so the emulsion capacity decreased. The ability of Na<sub>2</sub>CO<sub>3</sub> in the deacetylation reaction can be inhibited by the higher amount of OSA (Meng *et al.*, 2014).

### 3.2 Influence of sodium carbonate and octenyl succinic anhydride concentration on emulsion stability

The correlation between Na<sub>2</sub>CO<sub>3</sub> and OSA concentration on emulsion capacity is shown in Figure 2. The ANOVA result showed that the interaction between Na<sub>2</sub>CO<sub>3</sub> and OSA concentrations has a significant effect on KGOS emulsion stability ( $P < 0.05$ ). The addition of 2% Na<sub>2</sub>CO<sub>3</sub> and 4% OSA increased the emulsion stability up to 31.76%. Higher OSA concentration led to higher emulsion stability. However, at higher Na<sub>2</sub>CO<sub>3</sub> concentrations the emulsion stability decreased. This showed that the deacetylation effect is inhibited due to the higher amount of OSA. The stability of the KGOS emulsion is also due to the high viscosity of KGM (Xu *et al.*, 2015). Juntasakul and Maneintr (2018) reported that emulsion stability is affected by its viscosity. The viscosity was a crucial factor in maintaining the stability of the emulsion. The increase of viscosity of the aqueous phase in the oil in water emulsions, enhance the spatial repulsive force and electrostatic repulsion between the oil droplets and alter the rheological properties of the emulsion (Wang *et al.*, 2020).

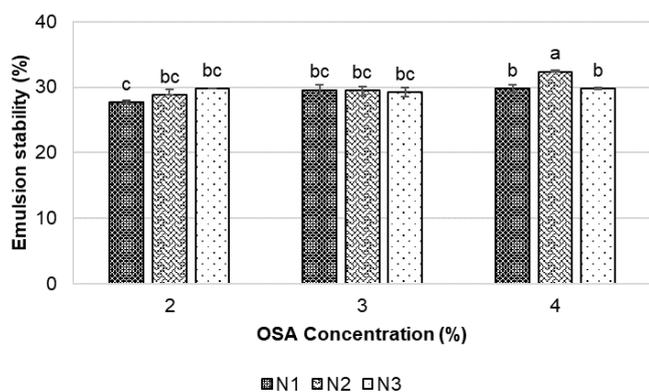


Figure 2. Effect of Na<sub>2</sub>CO<sub>3</sub> and OSA concentrations on emulsion stability. N1: 1% Na<sub>2</sub>CO<sub>3</sub>, N2: 2% Na<sub>2</sub>CO<sub>3</sub>, N3: 3% Na<sub>2</sub>CO<sub>3</sub>. Bars with different notations are statistically significantly different ( $p < 0.05$ ).

### 3.3 Characteristics of konjac glucomannan and konjac glucomannan

Table 1 shows the characteristics of KGM and KGOS. Modification of KGM can increase the capacity and stability of KGOS emulsion. Figure 3 shows the difference between KGM and KGOS. KGM has lower emulsion capacity and stability. It is caused by KGM's lack of lipophilic groups to stabilize oil droplets (Meng

*et al.*, 2018b) and the high viscosity of KGOS due to the limited movement of the droplets and re-agglomeration (Wang *et al.*, 2020). As shown in Figure 3, obvious oil zones could be seen in 3A and 3B (emulsion capacity and stability of KGM, respectively). In 3B and 3D, there was no oil zone, which indicated the best emulsibility for KGOS.

Table 1. Characteristics of konjac glucomannan (KGM) and octenyl succinic anhydride modified konjac glucomannan (KGOS)

Characteristics	KGM	KGOS
Emulsion capacity (%)	20.10±0.83	42.17±0.00
Emulsion stability (%)	14.97±0.73	31.76±1.14
Degree of substitution (%)	0.00±0.00	0.78±0.00
Contact angel (°C)	71.85±0.13	85.87±0.48
Viscosity (cP)	16573.33±80.83	18480.00±58.88

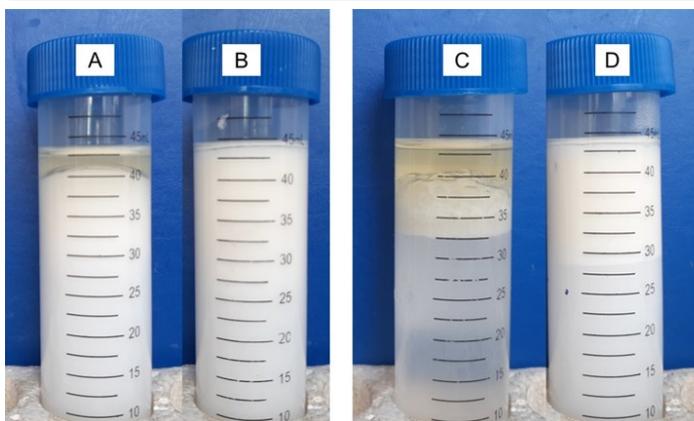


Figure 3. Emulsifying properties of konjac glucomannan (A) and octenyl succinic anhydride-modified konjac glucomannan (B) in oil-water (10 g: 40 g) emulsion. C and D were A and B incubated at room temperature for 30 days, respectively.

The increase in emulsion capacity and stability was caused by OSA esterification in KGM which was indicated by a higher degree of substitution up to 0.78%. The degree of OSA substitution in KGM was analyzed using HPLC. Figure 4 shows that OSA is seen at RT 4.5 mins. The equation obtained based on the standard curve results:  $Y = 163319x - 155526$ ,  $R^2 = 0.9972$ .

KGM and KGOS each have high viscosity. KGOS viscosity is higher than KGM (Table 1). OSA substitution in KGM increases the viscosity might be the more OSA addition weakened the deacetylation of the alkaline agent and then affected the viscosity KGOS (Meng *et al.*, 2018b). In addition, the esterified OSA in KGM causes an increase in molecular weight thereby increasing the viscosity of KGOS. Guo *et al.* (2021) reported that the viscosity is related to molecular weight, degree of acetylation, the particle size of the flour granules and surface morphology of the granules. Higher molecular weights were related to higher viscosities.

The contact angle is used to measure the film hydrophobicity (Wahyudi *et al.*, 2019). It is formed

between the liquid droplet and the solid surface. Contact angles are categorized into three groups, namely: 1) small contact angles (less than  $30^\circ$ ) correspond to the high wet ability of the film, 2) contact angles between  $30^\circ$  and  $90^\circ$  correspond to the partially wet ability of the surface, and 3) high contact angles (more than  $90^\circ$ ) indicate low wet ability (Naseri *et al.*, 2019). Table 1 and Figure 5 show that that KGOS has a higher contact angle than KGM. A higher contact angle value indicates an increased hydrophobicity character of the surface (Zhou *et al.*, 2009). The hydrophobicity increase in KGOS is due to the introduction of hydrophobic octenyl groups to the surface. The substitution of OSA groups can change the naturally hydrophilic starch to amphiphilic character and produce surface-active properties (Yusoff *et al.*, 2017; Altuna *et al.*, 2018).

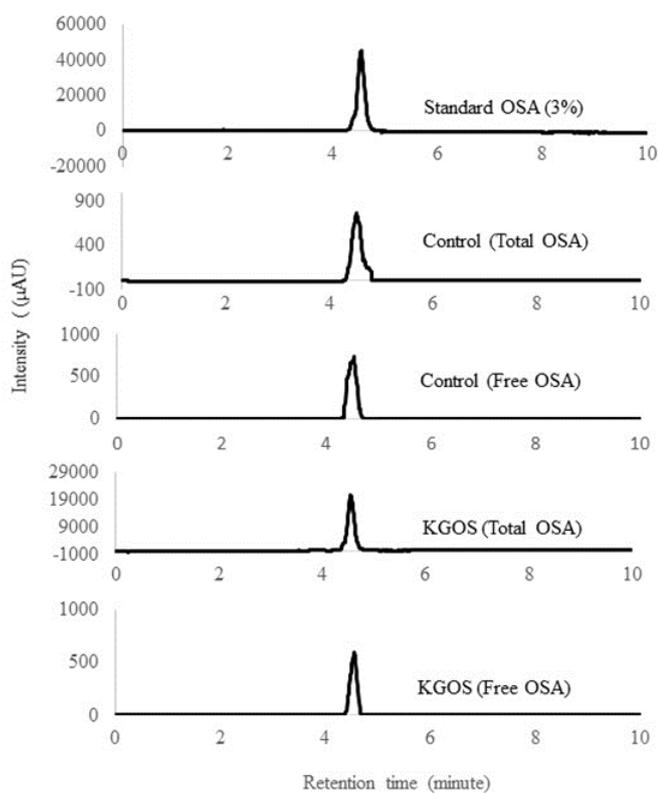


Figure 4. High-performance liquid chromatography/HPLC chromatogram of hydrolyzed OSA, Control (total OSA and free OSA), OSA modified konjac glucomannan/KGOS (total OSA and free OSA).

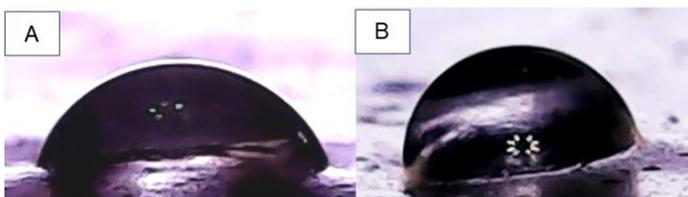


Figure 5. Contact angle konjac glucomannan/KGM (A) and octenyl succinic anhydride modified konjac glucomannan/KGOS (B).

### 3.4 FT-IR Analysis

The FT-IR spectra of the KGM and KGOS were

shown in Figure 6. The characteristic functional groups of KGM and KGOS were the same, and all produced absorptions characteristic of polysaccharides. The broad peak at  $\sim 3430\text{ cm}^{-1}$  corresponds to the stretching vibrations of O-H groups of the methyl in KGM. The peaks at  $\sim 2925\text{ cm}^{-1}$  were assigned to  $-\text{CH}_2-$  stretching vibrations. The peaks at  $\sim 1639\text{ cm}^{-1}$  belong to the intrinsic absorption of intramolecular hydrogen bonds and the  $-\text{CH}-$  peak appeared at  $\sim 1381\text{ cm}^{-1}$ . The peak at  $1060\text{ cm}^{-1}$  indicates the presence of a C-O group. Peaks attributed to  $\beta$ -glucosidic and  $\beta$ -mannosidic linkages were observed at groups at  $\sim 876\text{ cm}^{-1}$  and  $\sim 808\text{ cm}^{-1}$ , and the peak at  $\sim 1735\text{ cm}^{-1}$  assigned to the stretching of the C=O of the carbonyl of acetyl and OSA groups (Meng *et al.*, 2014; Meng *et al.*, 2018b). However, the intensity of the carbonyl peak in the KGOS spectra was slightly more intense than that of the KGM. The increase in the intensity of the KGOS carbonyl peak at  $1735\text{ cm}^{-1}$  is not obvious because the addition of carbonyl groups and deacetylation occurs simultaneously during esterification (Meng *et al.*, 2018b). Wardhani *et al.* (2017) reported that the acetyl group in glucomannan can be eliminated by  $\text{Na}_2\text{CO}_3$  (deacetylation), which then the OH groups on deacetylated glucomannan interacts with OSA through an esterification reaction (Sweedman *et al.*, 2013).

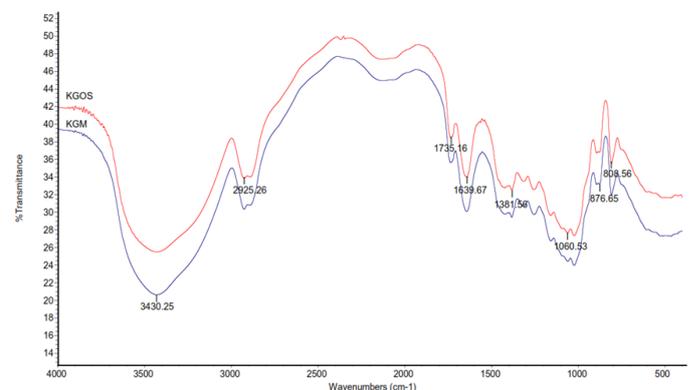


Figure 6. Fourier transform infrared (FT-IR) spectra of the konjac glucomannan (KGM) and octenyl succinic anhydride-modified konjac glucomannan (KGOS).

## 4. Conclusion

The concentration of  $\text{Na}_2\text{CO}_3$  and OSA had a significant effect on the emulsion capacity and emulsion stability. Modification of KGM with OSA was able to produce KGOS with amphiphilic properties characterized by increased emulsion capacity and stability. OSA has been esterified on KGM as indicated by an increase in viscosity, hydrophobicity, and degree of substitution as well as FT-IR analysis. Based on its characteristics, KGOS has the potential to be used as an emulsifier in the food industry.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

This research was funded by the Ministry of Research, Technology, and Higher Education, Republic of Indonesia grant number 8/E1/KPT/2021.

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