Synthesis of N-oleyl O-sulfate chitosan from methyl oleate with O-sulfate chitosan as edible film material

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Abstract. The research on the synthesis of N-oleyl O-sulfate chitosan through sulfonation reaction on chitosan with ammonium sulfate and followed by amidation reaction using methyl oleate has been done. In this study, chitosan was chemically modified into N-oleyl O-sulfate chitosan as an edible film making material. N-oleyl O-sulfate chitosan was synthesized by reaction between methyl oleate and O-sulfate chitosan. Wherein the depleted chitosan of O-sulfate chitosan into O-sulfatechitosan was obtained by reaction of sulfonation between ammonium sulfate and chitosan aldimine. While chitosan aldimine was obtained through reaction between chitosan with acetaldehyde. The structure of N-oleyl O-sulfate chitosan was characterized by FT-IR analysis which showed vibration uptake of C-H sp^3 group, S=O group, and carbonyl group C=O of the ester. The resulting of N-oleyl O-sulfate chitosan yielded a percentage of 93.52%. Hydrophilic-Lipophilic Balance (HLB) test results gave a value of 6.68. In the toxicity test results of N-oleyl O-sulfate chitosan obtained LC_{50} value of 3738.4732 ppm. In WVTR (Water Vapor Transmission Rate) test results for chitosan film was 407.625 gram/m^2/24 hours and N-oleylO-sulfate chitosan film was 201.125 gram/m^2/24 hours.

1. Introduction

Edible film is a thin layer serving as a packaging or food coating that can be eaten together with packaged products [1]. Moreover, Sanford and Hutchings (1987)[2]statedthat it is not only to extend the shelf life, edible films can also be used as carriers of food components, including vitamins, minerals, antioxidants, antimicrobials, preservatives, and ingredients to improve the taste and color of packaged products. In addition, the materials used to make edible films are relatively inexpensive, easily biologically modified (biodegradable), and simple manufacturing technology. The example of the use of edible films is in wrapping candy, sausage, fruit, and dried soup [3].

Indonesia as a maritime country certainly leaves waste in the form of shrimp shells. The main element of shrimp shells is chitin, a natural polysaccharide that has many uses such as chelating, emulsifying, and adsorbent. The properties of chitin are non-toxic and easily degradable. It encourages the modification of chitin with the aim of optimizing the usefulness as well as expanding the field of chitin application. One of the derivatives of chitin is widely developed because of its wide application is chitosan. Chitosan is a polysaccharide resulting from de-acetylation of chitin. Due to its biodegradable and non-toxic properties, chitosan are recommended to be used in environmentally friendly industry [4,5].

As aforementioned, the research on the production of packaging materials or food coatings that is environmentally friendly called edible film was conducted. Thus, by utilizing the chitosan characteristics as emulsifier to be made into N-oleyl O-sulfate chitosan by reacting sulfate chitosan with methyl oleate to form nontoxic and easily degradable product[6]. The results were characterized by using Fourier Transform-Infra Red (FT-IR) spectroscopy, Water Vapor Transmission Rate (WVTR) test, Hydrophilic-Lipophilic Balance (HLB) Test, and toxicity test by Brine Shrimp Lethality Test) BSLT method[7].

2. Materials and Methods

2.1. Instruments

The instruments used in this research were three-neck flask, burette (50 mL ± 0.05 mL), analytical balance, rotary evaporator, chemical glass 250 mL, drop pipette, hot plate and magnetic stirrer, ball condenser, volume pipette, measuring flask 250 mL, glass funnel, separating funnel, dropping funnel, measuring glass 10 and 100 mL, laboratory spatula, pipe $CaCl_2$, distilled water (*aquadest*) bottle, magnetic stirrer, stainless steel cylinder, thermometer, glass plate, desiccator, box, vial bottle, and infrared spectrophotometer.

2.2. Materials

The materials used in this research were Chitosan, alcohol 96%, sodium bicarbonate (pa), acetic acid (pa), oleic acid (pa), acetaldehyde (pa), sodium hydroxide (pellet), potassium hydroxide (pellet), anhydrous sodium sulfate, phenolphthalein, hydrochloric acid (pa), methanol, distilled water (*aquadest*), filter paper, toluene, oxalic acid (pa), H_2SO_4 (pa), *n*-hexane, and shrimp larvae.

2.3. Procedures

2.3.1. Synthesis of Chitosan Aldimine. A total of 3.0057 grams of chitosan were dissolved in 150 mL of 1% acetic acid, inserted into a three-neck flask connected with a water-flushed condenser. After soluble, 2 mL of acetaldehyde was added and then refluxed at room temperature for 6 hours while dripping 3 mL of glacial acetic acid through a dropping funnel.

2.3.2. Synthesis of O-Sulfate Chitosan. The chitosan aldimine obtained from the previous stage was added with 0.1 M ammonium sulfate with a ratio of 2:5 ($W_{kitosan}/V_{ammonium\ sulfate}$) and then put in shaker for 4 hours [8]. The compound was filtered with Whatman 42 filter paper, the precipitate was washed with distilled water (aquadest) until neutral and dried for 4 hours in an oven at 60 ℃.

2.3.3. Releasing Protective Group. A total of 3 grams of O-sulfate chitosan aldimine was added with sodium bicarbonate, 1:1, with toluene as solvent and sodium methoxide as catalyst at 60-70 ℃ for 3 hours. The result was washed with distilled water (aquadest) and filtered. Last, the precipitate was dried at 55 ℃.

2.3.4. Synthesis of Methyl Oleate. A total of 28.2 grams of oleate acid (p.a) were inserted into a flat bottom flask. Then, 14 mL of methanol and 50 ml of toluene (p.a) were added and stirred. Then slowly dripped 2 mL of $H_2SO_{4(c)}$ while cooled at 0 ℃ and refluxed at 80 ℃ for 5 hours. Then, it was put in the rotary evaporation. The residue was extracted with 200 mL n-hexane (technical) and washed with distilled water (aquadest). The top layer was taken and anhydrous Na_2SO_4 was added and allowed to stand for 30 minutes and filtered. The result of filtrate was put in rotary evaporation with temperature of 40-50 ℃.

2.3.5. Synthesis of N-Oleyl O-Sulfate Chitosan. A total of 1 gram of sulfate chitosan was put into a three-neck flask. Then, 3 mL of methyl oleate was added and stirred at 60-70 ℃ ± 3 hours. It was

filtered. Then, the precipitate was dried at room temperature. The results obtained were analyzed by FT-IR spectrophotometer.

2.3.6. Synthesis of Edible Film. A total of 1 gram of N-oleyl O-sulfate chitosan was dissolved in 15 mL 1% acetic acid. It was stirred for 30 minutes at 40 ℃. Then the mixture was poured into a glass plate whose sides are duct tape. The stainless steel cylinder was moved down to form a thin layer on the glass plate. It was dried at 25 ℃ for 24 hours.

2.3.7. Analysis of the Chemical Properties of N-Oleyl O-Sulfate Chitosan

2.3.7.1 Determination of Hydrophilic-Lipophilic Balance (HLB) Value. The analysis was done toward N-Oleyl O-Sulfate Chitosan. HLB was determined by using the formula as follow:

$$HLB = 20\left(1 - \frac{S}{A}\right) \quad (1)$$

Note: S=Saponification value
A=Acid number

2.3.7.2 DeterminationofWater Vapor Transmission Rate (WVTR) Value. The water vapor transmission rate of the film was measured by using gravimetric method, ASTM E96/E96M-10. The vapor absorbent material (silica gel) was placed in a vial bottle. Then the sample was placed in the mouth of the vial bottle covering the bottle. Later the end of the bottle was given glues so that no air enters.

The vial bottle was weighed with a precision of 0.0001 grams. It was placed in a closed container. It was given a small puddle of water. The bottle was weighed at the same hour (for 24 hours) and determined by the weight gain, the WVTR value is calculated by the formula:

$$WVTR = \frac{slope \times 100 \times 100\ m^2}{sample\ \ wide} = g/m^2\ /24\ hours\ \ (RH25^o\ C) \quad (2)$$

2.3.7.3 Toxicity Test by BSLT Method. Ten shrimp larvae in 100 µL of seawater were inserted into the test vial bottle, then 100 µL of sample solution was added. For each concentration, three repetitions are performed. The control was done with 100 µL blank solution then added seawater up to 100 µL. The observation was done after 24 hours by counting the number of alive and dead shrimp larvae, and then calculated its molarity like the following equation:

$$\%\ Mortality = \frac{Number\ \ of\ dead}{Number\ \ of\ dead + Number\ \ of\ alive} \times 100\% \quad (3)$$

3. Results and Discussion

3.1. O-sulfate chitosan

Chitosan used in this research is chitosan for synthesis. The FT-IR spectra used to produce absorption peaks in the numbers: 3448.72 cm^{-1}, 2924.09 cm^{-1}, 2846.93 cm^{-1}, 2337.72 cm^{-1}, 1635.64 cm^{-1}, 1427.32 cm^{-1}, 1381.03 cm^{-1}, 1319.31 cm^{-1}, 1257.59 cm^{-1}, 1080.14 cm^{-1}, 894.97 cm^{-1} (Figure 1).

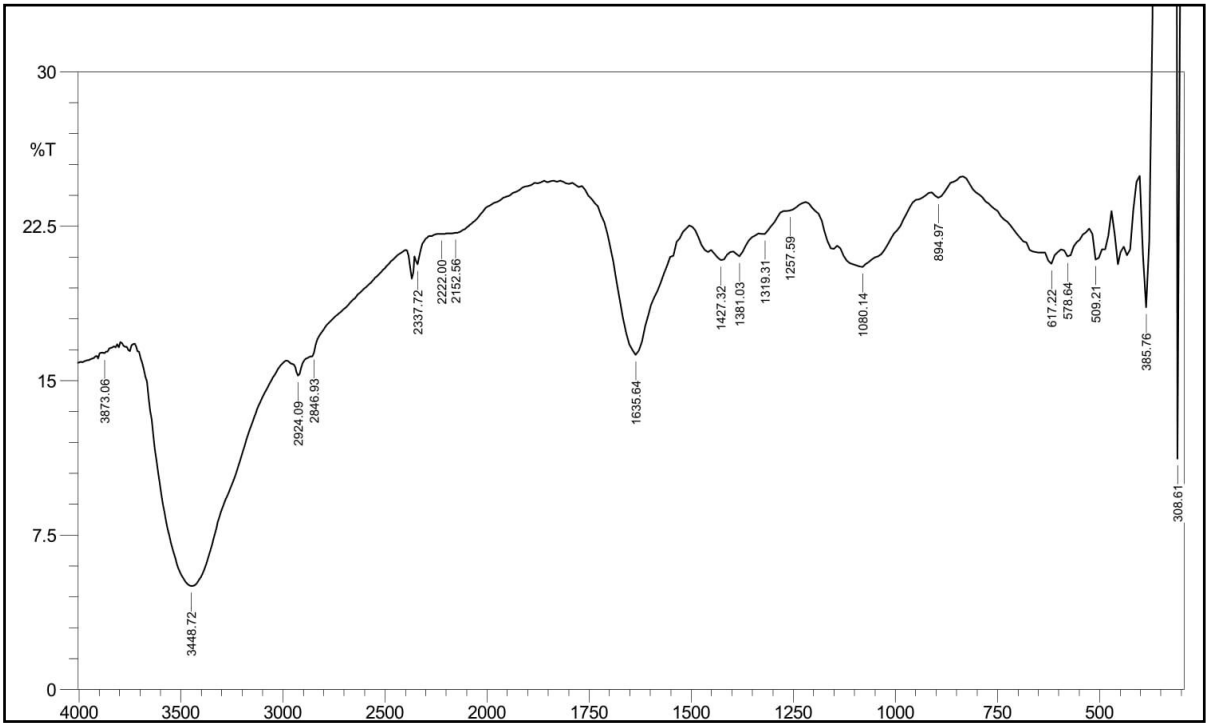

Figure 1. FT-IR spectra of chitosan

In this research, chitosan was first reacted with 1% acetic acid. The function of 1% acetic acid is as solvent for dissolving chitosan because chitosan is generally soluble in organic acids. One of them is acetic acid. A stirred situation was reacted with glacial acetic acid through a dropping funnel. The glacial acetic acid serves as a giver of acidic atmosphere in chitosan solution that was yellow. In a stirred state, an acetaldehyde solution was added to react with the chitosan solution.

In the preparation of chitosan sulfate, chitosan aldimine and ammonium sulfate served as a substitute that undergoes sulfonation reaction. H-SO_3^+ from ammonium sulfate substituted the CH_2O-H bonds in the O-sulfate chitosan to form the O-Sulfate chitosan aldimine with the ammonium (NH_3) and water (H_2O) as the byproducts. Ammonium and water were filtered using filter paper. It was washed with distilled water (*aquades*) to neutralize the chitosan aldimine that had been formed [9]. The conversion of chitosan aldimine to O-sulfate chitosan aldimine was essentially an electrostatic binding of sulfate ions in the chitosan reactive group (carbonyl group). This conversion was done to increase the adsorption capacity of chitosan due to the presence of sulfate ions, then the carbonyl group in chitosan will be more cationic. In addition, the presence of sulfate groups was expected to also bind the cationic groups. Sulfate ions were used because of their electrons. The reactivity of the O- sulfate chitosan is more stable than the chitosan because the carbonyl group has undergone permanent protonation with the attachment of sulfate ion. Then the deprotection against Aldine groups was done to bind to the N group on chitosan by using sodium bicarbonate. Sodium bicarbonate was added to O-sulfate chitosan aldimine to release the protective group with toluene as its medium and sodium methoxide as a catalyst.

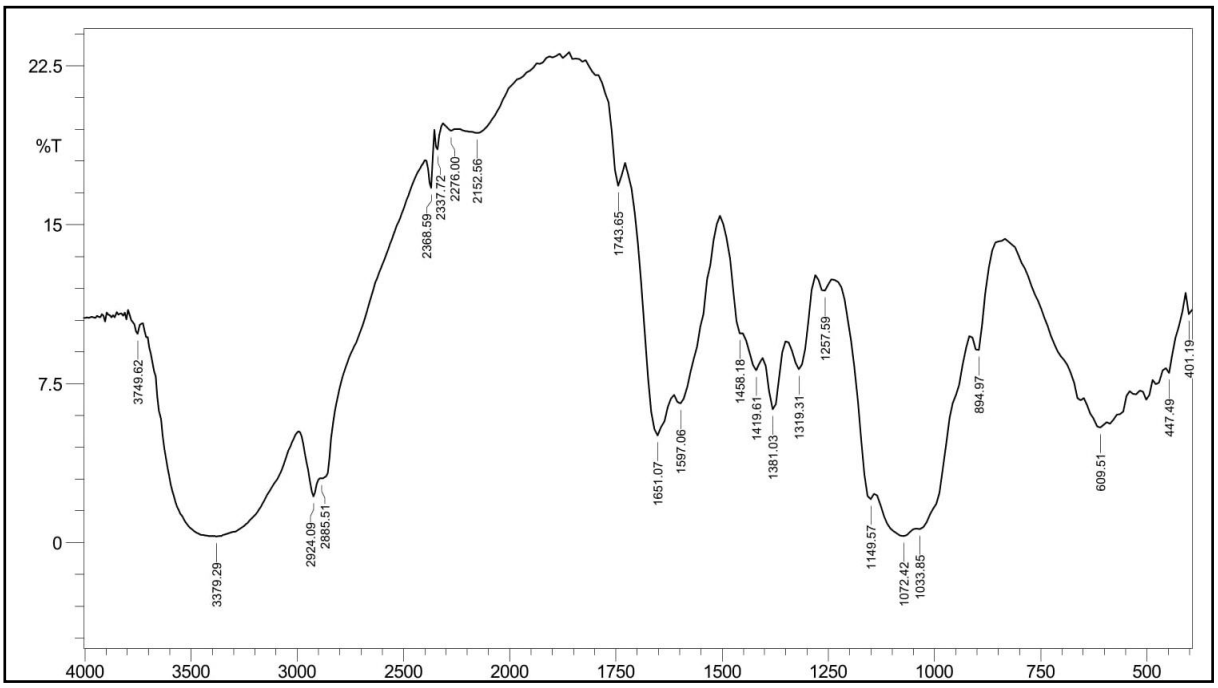

Figure 2. FT-IR spectra of O-sulfate chitosan

The FT-IR spectra of O-sulfate chitosan in figure 2 showed that the absorption peak in the wavenumber region 3379.29 cm^{-1} is the vibration of the O-H group. A stretching vibration of C-H sp^3 group was indicated on wavenumber 2924.09 cm^{-1} and 2885.51 cm^{-1}. It was supported by absorption peak at wavenumber 1419.61 cm^{-1} that is a bending vibration of C-H from CH_2 and spectrum 1319.31 cm^{-1} that is a typical absorption of C-H for CH_3.

The absorption peak at wavenumber 1651.07 cm^{-1} was a strain of the carbonyl group (C-O). It was also supported by the absorption peak at wavenumber 1072.42 cm^{-1} which was the vibration of the S = O group. It was the typical peak of O-sulfate chitosan [10].

The FT-IR spectra (Figure 2) showed the deprotection of protective group through the absorption peak at the wavenumber region 3379.29 cm^{-1} showing the overlapping vibration of O-H toward the N-H group of chitosan. The absorption peak in 2924.09 cm^{-1} and 2885.51 cm^{-1} showed the stretching vibration in C-H sp^3 and the absorption peak at the wavenumber 1597.06 cm^{-1} had weakened indicating deprecated imine.

3.2. Methyl oleate

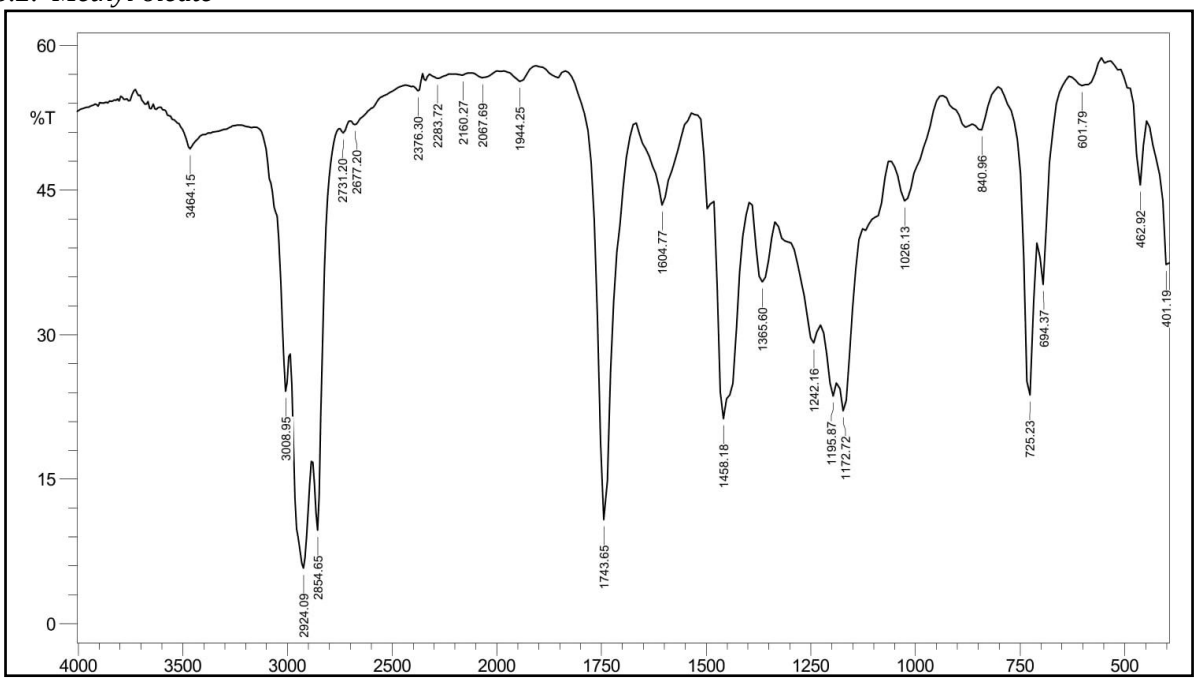

Figure 3. FT-IRspectraof methyl oleate

The FT-IR spectra in figure 3 showed the absorption peak at the wavenumber region 2924.09 cm^{-1} and 2854.65 cm^{-1}. According to Silvester, et al [10], it was the stretching vibration of C-H sp^3 supported by the absorption of bending vibration C-H sp^3 in wavenumber region 1458.18 cm^{-1}. The absorption peak at the wavenumber 1743.65 cm^{-1} was the absorption of the C=O carbonyl group of esters. It was formed and supported by the vibration of ester C-O-C in wavenumber region 1172.72 cm^{-1}. The spectra showed the absorption peak at wavenumber region 1604.77 cm^{-1} was the vibration of C=C alkene. Vibration absorption in wavenumber region 725.23 cm^{-1} was the rocking vibration of (CH_2) n at $(CH_2)_{14}$. In the FT-IR spectra of methyl oleate, the formed compound contained the C=O and C-O-C groups which were the characteristic of the ester. It did not contain the -OH group so that the compound was a methyl oleate compound.

3.3. N-oleyl chitosan O-sulfate

The FT-IR spectra in figure 4 showed the vibration peaks at wavenumber 2926.01 cm^{-1} and 2854.65 cm^{-1}. It was the stretching vibration of sp^3 supported by the absorption of bending vibration of C-H sp^3 at wavenumber 1450.47 cm^{-1}.The absorption peaks at wavenumber 1093.64 cm^{-1} and 1020.34 cm^{-1} were the vibration of the S=O group which was the typical peak of O-sulfate chitosan. The absorption peak at the wavenumber 1741.72 cm^{-1} was the absorption of the C=O carbonyl group of esters. It was formed and supported by the vibration of C-O-C ester in the wavenumber region 1170.79 cm^{-1}.The spectra showed the absorption peak at wavenumber region 1651.07 cm^{-1} was the vibration of C=C alkene. The absorption of stretching vibration at wavenumber region 3008.95 cm^{-1} supported by the bending vibration peak at wavenumber 725.23 cm^{-1} was the rocking vibration of $(CH_2)_n$ at $(CH_2)_{14}$. The FT-IR spectrum of N-oleyl chitosan O-sulfate contained a $(CH_2)_n$ group which was the characteristic of oleate. Thus, it can be concluded that the oleate in methyl oleate had entered and reacted with O-sulfate chitosan to form N-oleyl chitosan O-sulfate [10].

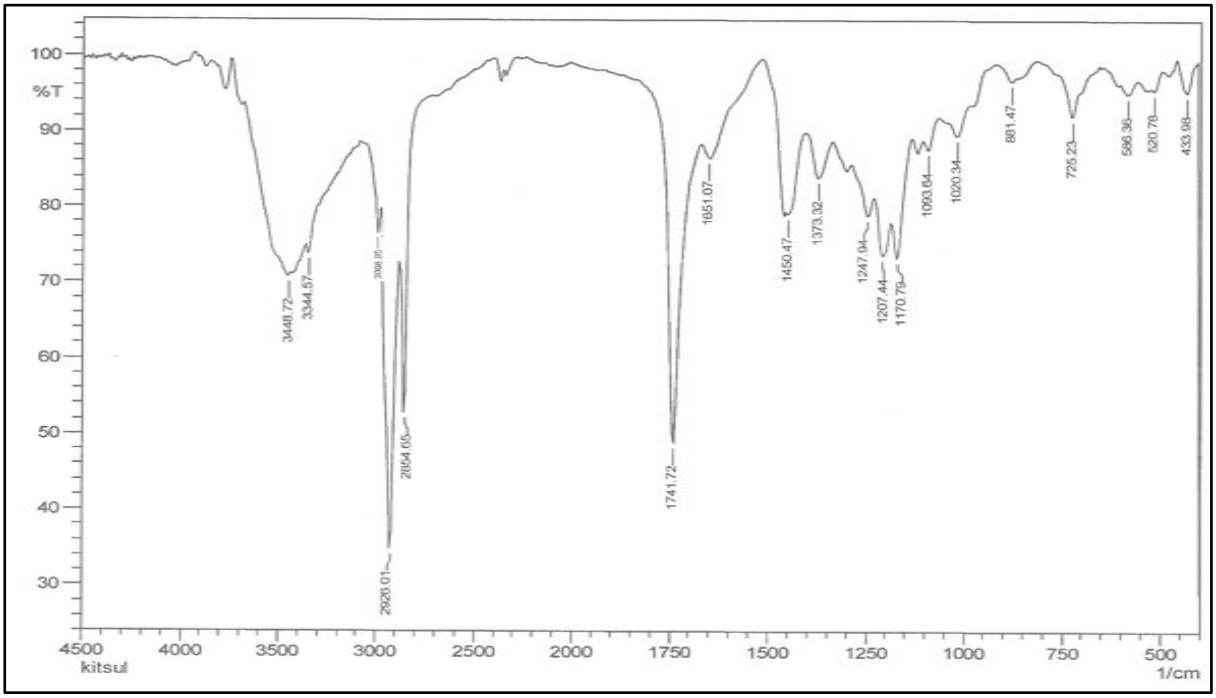

Figure 4. FT-IR spectra of N-oleyl chitosan O-sulfate

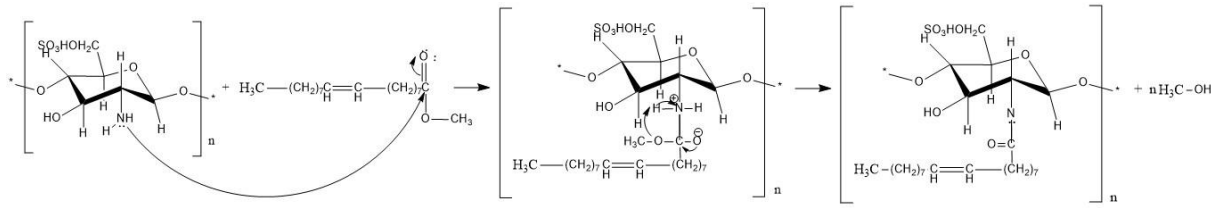

Figure 5. Mechanism reaction of N-oleylO-sulfate chitosan formation

3.4. Edible film

The produced edible film was transparent color, not rigid and elastic. Chitosan used as a base material gave transparent color to edible film. Then the oleate on the edible film provides elastic and non-rigid properties. The function of the use of oleate in the edible film was as plasticizer which was an organic material added to reduce the stiffness of the edible film. It had more flexible properties than the film without plasticizer.

Figure 6. *Edible film*

3.5. Hydrophilic-lipophilic balance (HLB) and saponification values

Table 1. Determination of acid number

Sample	Sample Mass (Gram)	Volume of Titration (mL)	Average volume of Titration (mL)	Acid number
N-oleyl O-sulfate chitosan	0.03	0.75	0.75	14.02
	0.03	0.75		
	0.03	0.75		

Table 2. Determination of saponification value

Sample	Sample Mass(Gram)	Volume of Titration (mL)	Average volume of Titration (mL)	Saponification value
Blank		20.75	20.75	
		20.75		
		20.75		
N-oleyl O-sulfate chitosan	0.03	19.50	19.75	18.7

The HLB test gave the data of acid number and the saponification value from N-oleylO-sulfate chitosan. The acid number was 14.02 and the saponification number was 18.7. Thus, HLB value obtained from N-oleyl O-sulfate chitosan was 6.68.

The HLB value of N-oleylO-sulfate chitosan was 6.68. If it was viewed by a scale showing the surfactant function of the HLB value was at position 4-7 indicating that the compound can be used as a W/O emulsion.

3.6. Water vaportransmission rate(WVTR) test

Table 3. Determination of water vapor transmission rate

Sample of Film	Silica gel Mass + Vial bottle (gram)			Time used (hour)	Sample area (m^2)
	G1	G2	G		
Chitosan	11.7426	12.3948	0.6522	24	16.00
N-Oleyl O-Sulfate Chitosan	9.2521	9.5739	0.3218	24	16.00

The WVTR was used to determine the permeability value of a material toward water vapor. Water vapor permeability is a measure of a material as it can be passed by water vapor.

$$WVTR = \frac{24 \times 10}{A} \times \frac{W}{t}(gram/m^2/24\ hours)$$

Water vapor movement in a food product can occur when the difference of water activity (aw) happen. The difference of the humidity of the food product with its surrounding environment can be controlled by regulating the aw on the food, or by wrapping it with an edible film layer which had good barrier properties against the water vapor transmission and can avoid the loss of water vapor effectively. Water vapor transmission was strongly influenced by the temperature, thickness, type and the concentration of the plasticizer and the nature of the material to form edible film.

The data showed that the permeability of water vapor from the chitosan film was relatively high if it was compared to N-oleyl O-sulfate chitosan film. It was because of the hydrophilic properties

possessed by the chitosan. The chitosan polymer of it had a large hydrogen bond so that the water vapor permeability value was high. However, the chemical polymer component of N-oleyl O-sulfate chitosan had non-polar lipid that had lower water vapor permeability that made the film can retain the water.

3.7. Toxicity test by BSLT (Brine Shrimp Lethality Test)

The observation of bioactivity potential was done based on lethal concentration 50% (LC_{50}) value. It was a value that showed the concentration of toxic substances that can cause the death of organisms up to 50%. Chemical compounds belonged to potentially active or toxic when having LC_{50} less than 1000 ppm, otherwise, it is not toxic if it has LC_{50} value more than 1000 ppm.

Based on the molarity test, by using SAS Probit Analysis, of shrimp larvae from N-oleyl O-sulfate chitosan, the LC_{50} value was 3738.4732 ppm. The value indicates that N-oleyl O-sulfate chitosan is non-toxic. It happened because the sulfate used in the synthesis of N-oleyl O-sulfate chitosan was not a concentrated sulfuric acid, but ammonium sulfate which tends to be lower level of toxicity, in addition of the ammonium sulfate used had a low concentration of 0.1 M.

4. Conclusions

Based on the results, it can be concluded that N-oleylO-sulfate chitosan can be synthesized by sulfonation reaction in chitosan and ammonium sulfate and followed by amidation reaction using methyl oleate.N-oleyl O-sulfate chitosan can reduce the rate of water vapor transmission in producing edible film through Water Vapor Transmission Rate (WVTR) analysis. In addition, N-oleyl O-sulfate chitosan can be used safely in producing edible film. It can be seen through the toxicity test by BSLT Method that shows that the value of LC_{50} was 3738.4732 ppm.

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