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Optimation of Alkoxide formed step on Carboxymethyl Kappa Carrageenan synthesis

Irma K Kusumaningrum[1*], Anugrah R Wijaya[1], Siti Marfuah[1] and Mira Nur Fadilah[2]

[1]Universitas Negeri Malang
[2]Institut Teknologi Sepuluh Nopember

*E-mail: irma.kartika.fmipa@um.ac.id

Abstract. Carboxymethylation of *kappa* carrageenan with mono-chloroacetic acid as a reactant had been synthesized to produce carboxymethyl *kappa* carrageenan (CMKC). Carboxymethylation of *kappa* carrageenan was obtained in two steps. First, alkalization of *k*-carrageenan with NaOH to create alkoxy k-carrageenan, and optimization of the alkalization step, the optimization had been done include the mass ratio between NaOH and *k*-carrageenan and alkalization's temperature, second, etherification of alkoxy *k*-carrageenan with Monochloroacetic acid (MCA) as etherification reactant, to produce CMKC. The optimal condition (2) characterization of the CMKC properties, including the degree of substitution (DS) and FTIR spectrum. The optimal condition was based on the formed degree of substitution(DS) of CMKC. The alkalization of k-carrageenan has been performed on three variations of mass ratio between NaOH: *kappa c*arrageenan flour that dispersed in isopropanol, and then the mass ratio of NaOH: k-carrageenan that has the highest DS was used in the experiment to find the optimal alkalization temperature. To find the optimal temperature, the alkalization was performed at, 40^{o}, 55^{O} and 70^{o}. The CMKC that has the highest DS value was produced when the mass ratio between NaOH mass: *kappa* carrageenan is 7,2:1, and the temperature of alkalization is 70^{o}C, The DS value that reached when the CMKC synthesis had been done on the optimal alkalization condition is, 0,85.

1. Introduction

K-carrageenan is a polysaccharide that is extracted from *Euchema cottoni*, *k*-carrageenan is a type of carrageenan that has the highest gelling power formed among the other types of carrageenan. The use of polysaccharides, including k-carrageenan, is extensive, the polysaccharide is frequently used as viscosity regulator, binder and mass forming, the nature of polysaccharide is influenced by their structure.

The modification of polysaccharides structure can occur n many ways, carboxymethylation of polysaccharide is done to convert the hydroxyl group to carboxymethyl group, this conversion is done to change the chemical properties and increase its interaction with the polar environment. Many researchers have carboxymethylated some of polysaccharides before, one of the most widely used of polysaccharide carboxymethylation results is carboxymethyl cellulose (CMC) [1]. The number of hydroxyls (–OH) groups that can be substituted is expressed by the degree of substitution (DS). The DS value could determine the success of the carboxymethylation process. Lawal (2008) has applied many carboxymethylation reactions to increase the value of the DS of the carboxymethylated product of polysaccharide. The value of DS affect by some reaction conditions, the mass ratio between etherification agent and alkali mass ratio to polysaccharide mass, reaction temperature, reaction medium

and reaction time [2]. The reaction conditions of the carboxymethylation process vary depending on the type of polysaccharide, it is necessary to determine the optimal conditions of carboxymethylation every kind of polysaccharide, by research.

Hydroxyl group of k-carrageenan can be converted to the carboxymethyl group, the carboxymethylation of k-carrageenan produced carboxymethyl k-carrageenan (CMKC). Synthesis of CMKC had been done by the other researcher before [3]. The carboxymethylation process consists of two stages, alkoxide formed and etherification. The alkoxide formed step converts k-carrageenan to alkoxy k-carrageenan, etherification step converts alkoxy- k- carrageenan to carboxymethyl k-carrageenan (CMKC). Products of carboxymethylation process of k-carrageenan have a higher solubility in water compared to k-carrageenan, because the carboxymethylation process changes the hydroxyl group becomes a carboxymethyl group that has higher interaction power with the polar environment than hydroxyl groups [4].

Optimization of CMKC synthesis process is important to be done, in order to produce CMKC that has a high value of DS, multiple carboxymethylation enhance the DS value, but this procedure is not efficient. In this study, the effort to increase the DS value had been made by optimizing the reaction temperature, and the mass ratio between NaOH to the k-carrageenan mass of alkoxide formed step. The optimal condition of the reaction is determined by the highest of DS value that is achieved and the appearance of hydroxyl and carboxyl functional groups peaks on the FTIR spectrum of k-carrageenan and CMKC.

2. Materials and Methods

2.1. Optimization of carboxymethyl k-carrageenan synthesis

The CMKC synthesis method refers to [5] with modifications. 1g of k-carrageenan flour is suspended in 25 mL of isopropanol and stirring for 15 minutes at room temperature, then 3 ml 96% ethanol was added, and the mixture was stirred for 10 minutes. Later NaOH granules (the mass variations are (1.5 g; 6 g; 7.2 g)) were added, and the mixture was stirred for 1 hour at the specific temperature (40^{o}C, 55 ^{o}C, 70 ^{o}C), later the mixture reacted with 0.8 g of mono-chloroacetic acid that is dissolved in 10 ml of 96% then the mixture was stirred for 4 hours, at 70^{o}C, to produce CMKC. The CMKC produced was filtered, soaked in ethanol and neutralized with glacial acetic acid. The neutralized CMKC has washed again in ethanol, filtered and dried at 50^{0}C.

2.2. FTIR spectrum analysis

FTIR spectrum analysis was carried out on the FTIR spectrum of the mix of k-carrageenan and CMKC dry powder that had been mixed with KBr (1:10 g / g). The wave numbers range that is analyzed is 4000 cm-1 to 400 cm-1region by using a spectrophotometer Fourier Transform Infra-Red (FTIR) Prestige 21 Shimadzu.

2.3. Determination of degree of substitution (DS)

DS value is determined by titrimetric method and is calculated with the below formula

$$DS = \frac{\text{MR x M (B}-\text{S)}}{1000\text{W}}$$

W : sample weight (gram)
B : volume of HCl 0,13 M (for blank titration) (mL)
S : volume of HCl 0,13 M (for sample titration) (mL)
M : Molarity of HCl (mol/L)
MR : Mass weight of k-carrageenan monomer (g/mol)
DS :Degree of substitution

Titration is carried out in a blank solution and CMKC solution. As a blank, alkoxy k-carrageenan is used.

2.4. Preparation of blank solution and sample solution for DS test

Alkoxide of k-carrageenan (for blank solution) or CMKC (for CMKC solution) weighed 0.1 g then added with 10 mL 0.12 NaOH, then stirred with a magnetic stirrer at room temperature for 30 minutes, later the mixture is combined with methyl red indicator. The excess of NaOH is titrated with 0.13 M HCl.

3. Result and Discussion

Carboxymethyl kappa-carrageenan (CMKC) is a k-carrageenan derivative that is made through the substitution process of hydroxyl groups of the k-carrageenan polymer chain. There are two reaction steps in the synthesis of CMKC. The first step is the alkoxide formatted step of k-carrageenan. K-carrageenan powder was suspended in an isopropanol-ethanol mixture as a reaction medium. The reaction of alkoxide formation is carried out in a semi-polar alcoholic medium, the polarity of the media influences the repelling force between the negative charges of polysaccharide chains that have negatively charged hydroxyl groups, the more polar media is used, the repelling force between the polymer chains is higher, facilitate the swelling, so that the diffusion of reagents into the polysaccharide chain will take place more easily [6].

K-Carrageenan is a polymer that has many hydroxyl groups, which causes it to have a weak acidic characteristic. The presence of NaOH in the reaction system will pull the H^+ from the hydroxyl group of k-carrageenan and then bind to the OH^- ion of sodium hydroxide, while the Na^+ ion of NaOH binds to the O^-atom of k-carrageenan. The changes of k-carrageenan to alkoxy k-carrageenan causes their solubility in water increase

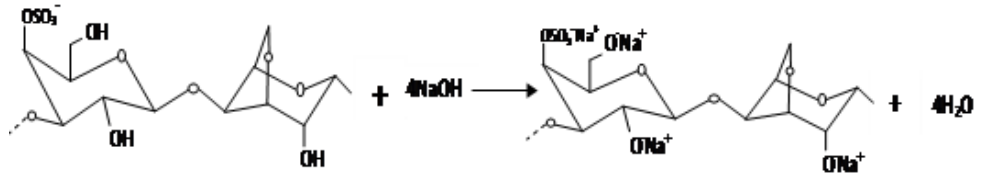

Figure 1. The proposed of reaction equation between k-carrageenan and NaOH

Beside the hydroxyl group, k carrageenan has a sulfate group in their chain, the probability of side reactions between sulfate groups and NaOH is large because sulfate groups have a higher acidity than hydroxyl groups. Therefore it is possible that the reaction between the sulfate group and NaOH is easier than the reaction between the hydroxyl group and NaO if the mass ratio between NaOH and k-carrageenan is too small. It is possible that the amount of NaOH that was used to convert the sulfate group of k-carrageenan into sodium salt is more than the amount of NaOH that was used to form alkoxy of k-carrageenan, thus reducing the formation of alkoxy-k carrageenan. The reaction between NaOH and k-carrageenan will produce an alkoxy group of k-carrageenan, an increase in the mass ratio of NaOH to k-carrageenan should increase the number of OH groups converted to alkoxy k-carrageenan formed. The success of the formation of alkoxy carrageenan determines the formation of carboxymethyl groups in carrageenan, the more hydroxyl groups are converted into carboxymethyl groups, the higher the DS value, the more NaOH that is used the chance of hydroxyl group converting to carboxymethyl group is greater. However, increasing the amount of NaOH also increases the probability of a side reaction that produces sodium salt from k-carrageenan as an unwanted byproduct. No data has been found about the stability constants of the alkoxy formation of k-carrageenan and the formation of sodium k-carrageenan, that can be used to estimate which reactions are more likely to occur between the two. It is estimated that an increase in the mass ratio of NaOH to the mass of k-carrageenan will increase the value of the degree of substitution. However, because NaOH is easy to attract water when the mass ratio of NaOH: k-carrageenan is higher, the system tends to hold water more and dissolve the CMKC which is soluble in the water, so the use of more NaOH potent to reduce the CMKC mass obtained. The much of NaOH was used tends to cause the termination of the k-carrageenan polymer chain so the CMKC solution will be decreased.

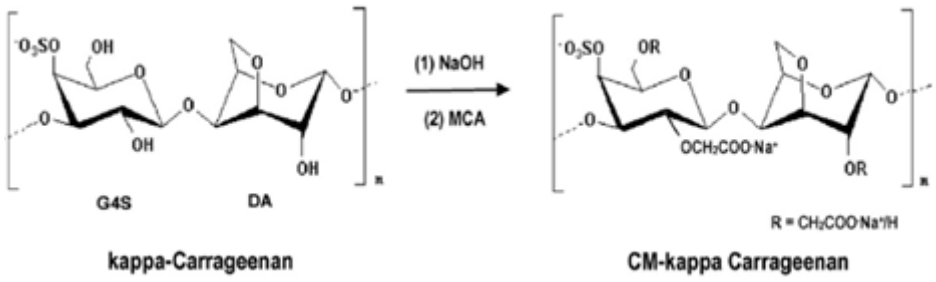

Figure 2. Etherification reaction to produce Alkoxy k-carrageenan

Alkoxy k-carrageenan that was produced then convert to CMKC by etherification reaction, with MCA as a reactant. Alkoxy k-carrageenan is reacted with mono-cloroacetic acid to produce CMKC. Side reaction between NaOH and mono-chloroacetic acid maybe occurs in this step, the solubility of mono-cloroacetic acid in water is higher than the alkoxy-carrageenan solubility, the more water in reaction medium will increase the chance of side reactions between NaOH and mono-cloroacetate. The occurrence of this side reaction causing a reduction in the number of available mono-cloroacetic acid molecules to react with the hydroxyl group of alkoxy k-carrageenan thus causing the number of hydroxyl groups that was converted to carboxylate decrease.

The Degree of Substitution (DS) is the average value of the carboxyl group present in each monomer unit. The degree of substitution correlates with the success of the hydroxyl group substitution process by carboxyl groups, the higher the DS value, the more substituted hydroxyl groups. Based on the experiment, the CMKC that was produced on the mass ratio between k-carrageenan and NaOH is 1:7,2 and the alkoxy formed reaction temperature is 40^{o}C, has the highest DS, then the optimal alkoxy temperature determination experiment was done on the mass ratio between k-carrageenan and NaOH IS 1:7,2. Alkoxy temperature effect on the possibility of alkoxy k-carrageenan. Inter-molecular chemical elimination of cellulose's hydroxyl group increases when the reaction temperature increase [7].

Table 1. The DS Value of CMKC that was produced on the various mass ratio between k-carrageenan and NaOH and various alkoxy formed temperature

NaOH mass (g)	Monochloroacetic mass (g)	The temperature of Alkoxide formed step	The temperature of etherification step	DS
1,5	0,8	40	70	0.45
6	0.8	55	70	0,50
7.2	0,8	70	70	0.85
7.2	0,8	70	70	0.40
7.2	0,8	70	70	0.50
7.2	0,8	70	70	0.85

The carboxylate formed can be seen through the comparison between the FTIR spectrum of carrageenan, Na-carrageenan, and CMKC shown in Figure 1.

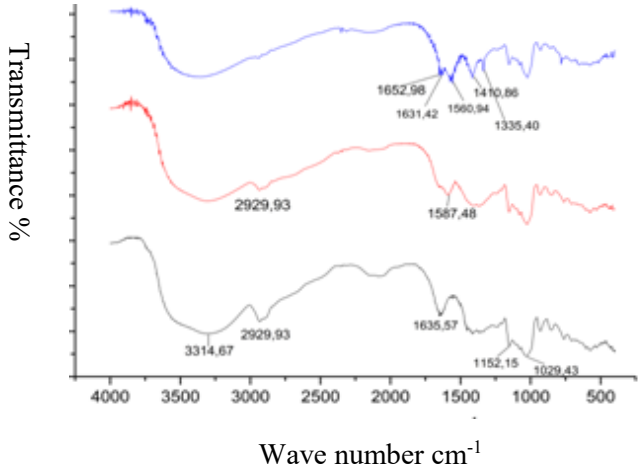

Figure 3. FTIR spectrum of Carboxymethylation of k-carrageenan

Carboxymethylation of k-carrageenan produces a new absorption band which shows the presence of carboxyl groups. The reduced absorption intensity at wave numbers around 3300 cm^{-1} and 2929.43 cm^{-1} occur due to the decreasing C-H and O-H stretch due to the substitution of hydrogen atoms on hydroxyl groups of k-carrageenan into COO^- groups. This process is reinforced by the appearance of absorption bands at wave numbers 1652, 56 cm^{-1} and 1410 cm^{-1} that suitable with a typical area of carboxyl group peak, whereas 1700-1650 cm^{-1} and 1425-1410 cm^{-1} for asymmetric and symmetric COO- vibration.

4. Conclusion

Carboxymethyl k-carrageenan can be synthesized using an etherification agent of mono-chloroacetic acid (MCA). Typical uptake in FTIR spectrum, which marks the formation of CMKC is absorption at wavenumbers 1652,56 and 1410 cm^{-1}. The reaction temperature of the alkoxy-carrageenan formation stage and the NaOH mass ratio to k-carrageenan affect the value of DS.

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