# Effect of H2O2 Concentration on Molecular Weight and Functional Properties of Sulfated Polysaccharides from Red Seaweed (Kappaphycus alvarezii)

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# Effect of $H_2O_2$ Concentration on Molecular Weight and Functional Properties of Sulfated Polysaccharides from Red Seaweed (*Kappaphycus alvarezii*)

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**Abstract.** κ-carrageenan is sulfated polysaccharides (SPs) extracted from red seaweed (*Kappaphycus alvarezii*). κ-carrageenan is sulfated anionic polymers composed of D-galactose units linked alternately with α-1,4 and β-1,3 linkages. Low molecular weight (LMW) of κ-carrageenan has large applications in pharmaceutical and biomedical function. In this study, κ-carrageenan was degraded by oxidative methods in the presence of hydrogen peroxide ($H_2O_2$). The average molecular weight of degraded κ-carrageenan was measured by intrinsic viscosity methods with the Mark Hauwink equation. The aimed of this research was to study the effect of hydrogen peroxide concentration (0.5-1.5% w/v) on the functional properties and the molecular weight of κ-carrageenan. The condition reaction was kept at pH 7 and temperature 45°C. These results showed that the degradation rate of κ-carrageenan was positively related to $H_2O_2$ concentration. The extent of degradation of κ-carrageenan was obtained the amount of 56.5% with operation conditions of 1.5% $H_2O_2$ concentration for 20 minutes. The functional properties of the molecular weight of κ-carrageenan were characterized by Fourier transform infrared spectroscopy (FT-IR). FT-IR analysis of treated κ-carrageenan showed that the degradation of κ-carrageenan in the presence of $H_2O_2$ caused a slight decrease in sulfate content.

**Keywords**: κ-carrageenan, degradation, hydrogen peroxide, molecular weight.

## INTRODUCTION

κ-carrageenan is a sulfated galactan extracted from red algae (Rhodophyceae), which is abundantly available in Indonesian waters. The basic structure of κ-carrageenan is ammonium sulfate ester of D-galactose polymer linked in α-1,3 and β-1,4 positions [1]. κ-carrageenans are increasingly used in food industry applications as stabilizing or texturing agents [2-3] and most recently used in the pharmaceutical industry as an excipient in pill and tablets [4]. Properties of the κ-carrageenan not only on its structure but also on molecular weight. Generally, κ-carrageenans have a high molecular weight and it makes limited on their applications.

Attempts have been conducted to broaden the utilization of κ-carrageenan, one of which is through degradation of molecular weight and maintained the sulfate content. The degradation process of κ-carrageenan can be studied with some of the processes, such as using enzymatic methods [5-6], hydrolysis under mild acid [7-11], irradiation [12-13], microwave-assisted [14], ozonation [15] and ultrasonication process [16-18]. Some of the degradation methods have a weakness, degradation of κ-carrageenan by enzymatic either using specific or non-specific enzymes, it is a relatively

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expensive and complex process [19-20], by the ozonation the equipment for processing is complex and expensive [21]. Using hydrogen peroxide as an oxidizing agent able to enhance the degradation of κ-carrageenan easy process and created no harmful by-product [22-23]. This chemical is therefore considered more environmentally friendly and is preferred especially when a chlorine-free process is desired [24 & 5]. The combination can also be recommended. According to Hien et al. [15] & Prajapat et al. [26] studied the degradation of chitosan by combination radiation with hydrogen peroxide. When used only gamma radiation only slightly influence to the reduction in chitosan molecular weight the presence of hydrogen peroxide can increase the extent of the degradation of chitosan.

There are several reports of degradation polysaccharide in the presence of $H_2O_2$. However, the research examining the degradation of κ-carrageenan with hydrogen peroxide oxidation is limited and only a few of the reports already exist. Therefore, the aims of this work is to study the effect of hydrogen peroxide concentration on the functional properties of molecular weight of κ-carrageenan during $H_2O_2$ treatment.

## METHODS AND MATERIALS

### Preparation of κ-Carrageenan Solution

The raw material used in this work was commercial κ-carrageenan derived from seaweed *kappaphycus alvarezii*, produced by CV. Karagen Indonesia, Semarang, Indonesia. For preparation, the κ-carrageenan was dissolved in distilled water at 70 °C and stirred for 15 minutes. Purified κ-carrageenan was obtained by filtration and isopropyl alcohol (E. Merck Cat. No. 109634.2500) precipitation. The pH of κ-carrageenan solution was adjusted by adding HCl with 37% of purity (E. Merck Cat. No. 100317) or NaOH with > 99% of purity (E. Merck Cat. No. 104698). All chemical reagents were of analytical grade and directly used without further purification.

### Degradation by $H_2O_2$

One hundred milliliters of κ-carrageenan solution were added $H_2O_2$ solution with different concentration (0.5%; 1% and 1.5% w/v). The reaction was maintained at temperature 45°C and pH 7 for all treatments, the oxidation reaction in the presence of $H_2O_2$ was carried out within 20 minutes of reaction. Every 5 minutes, the degraded carrageenan was taken for analyzed the intrinsic viscosity using Ubbelohde viscometer.

### Molecular Weight Determination

For determination of the molecular weight of $H_2O_2$ treated κ-carrageenan, 5 different concentration (0.0625 to 1.0 % w/v) of $H_2O_2$ treated κ-carrageenan solution were prepared. A portion of buffer solution pH 7 was added to adjust polysaccaharide concentrations and to keep polysaccharide molecules from intermolecular aggregation [15]. The efflux times of the solutions were measured using an Ubbelohde capillary viscometer (type 531 030c Schott-Generate, Germany) at a constant temperature at 45.0±0.1 °C. The intrinsic viscosity ([η]) was calculated from the spesific viscosity ($\eta_{sp}$). The intrinsic viscosity is the average intercept of Huggins and Kraemer equation [28] in Equation (1).

$$\frac{\eta_{SP}}{c} = [\eta] + k_H[\eta^2] \quad (1)$$

In this equation, $\eta_{sp}$, ([η]), $k_H$, and c are specific and intrinsic viscosity, Huggins constant, and the concentration of the solution, respectively. The specific viscosity ($\eta_{sp}$) and the Huggins constant ($k_H$) are dimensionless, while the intrinsic viscosity ([η]) and the concentration (c) have the units of $mL.g^{-1}$ and $g.mL^{-1}$, respectively. The value of $k_H$ for the κ-carrageenan solution is 0.35 [28].

The molecular weight of κ-carrageenan ($M$) was calculated from the intrinsic viscosity data by Mark Houwink equation (equation 2)

$$[\eta] = k_{MH}M^{a} \quad (2)$$

In this equation, $k_{MH}$ and $a$ are constants for a given system. In this work, the values of $k_{MH}$ and $a$ of κ-carrageenan are 0.00598 and 0.90, respectively. The symbols of $M$ and $[\eta]$ are expressed in $g.gmol^{-1}$ and $mL.g^{-1}$, respectively [29].

## Functional Group Analysis

The functional group analysis were determined using Fourier Transform Infra-red spectroscopy (FT-IR). FT-IR analysis was recorded in powder form by using PerkinElmer Spectrum IR 10.6.1, USA.

## RESULTS AND DISCUSSION

### Effect of $H_2O_2$ Concentration on Molecular Weight

Fig.1 shows the change in Mw of κ-carrageenan as the concentration of $H_2O_2$ was varied from 0.5% to 1.5% w/v and the reaction time was varied from 0 min to 20 min. In the first 5 minutes, Mw was reduced sharply from 423.82 kDa to 260 kDa, approximately. After the 5 minutes, Mw changed only slightly. The fenomena of this work can be seen that the higher concentration of $H_2O_2$ able to increase the extent of degradation of κ-carrageenan. In the 20 minutes of reaction time, the Mw are approximately 232.8 kDa, 214.1 kDa, and 184.5 kDa, corresponding to original $H_2O_2$ concentrations of 0.5%, 1%, and 1,5%, respectively. The effect of $H_2O_2$ concentration on reduction of molecular weight of κ-carrageenan during $H_2O_2$ treatment depicted in Figure 1.

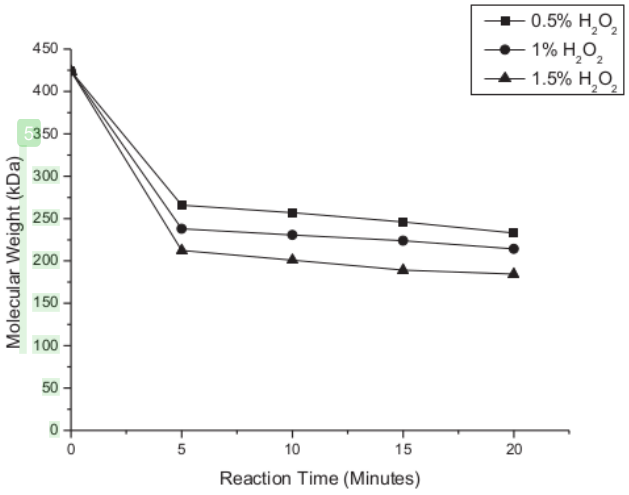


**FIGURE 1**. Effect of $H_2O_2$ concentration on depolymerization of molecular weight of κ-carrageenan. Reaction was kept at 29±1 $^0$C (■: 0.5%; ●: 1%; ▲: 1.5%)

The degradation of κ-carrageenan used chemical treatment can be an effective method to reduce the molecular weight of polysaccharide, as chitosan [30], alginate [31], and fucoidan [32]. Li et al. [31] reported that the effect of increasing $H_2O_2$ concentration able to increase to the extent of alginate depolymerization. The used of $H_2O_2$ concentration of 0.25% can the reduce the molecular weight of alginate from 230 kDa to 143 kDa with a value of the extent of degradation of 37.8%. Hou et al. [32] also reported that the increasing of $H_2O_2$ concentration able to reduce the molecular weight of fucoidan. During $H_2O_2$ treatment at 50$^o$C, 0.4 M of $H_2O_2$ concentration and reaction time 6 h able to reduce the molecular weight of fucoidan from 58 kDa to 2.6 kDa. It all reported can demonstrate that the concentration of $H_2O_2$ is an important factor for the degradation of κ-carrageenan.

The decreasing of molecular weight of polysaccharide by $H_2O_2$ is considered to be caused predominantly by free radical [31]. The resulting radicals are powerful oxidants and capable of abstracting hydrogen atoms from the glycosidic bonds of alginate, rearranging the structure of the molecular and breaking glycosidic bonds [33]. Tian et al. [34] studied the mechanism of the depolymerization of chitosan by $H_2O_2$. In the depolymerization system of chitosan with $H_2O_2$ can be shown in Eqs. (3) and (4), and the total reaction is shown in Eqs. (5).

$$R - NH_2 + H^+ = R - NH_3^+ \quad (3)$$
$$H_2O_2 = H^+ + HOO^- \quad (4)$$
$$H_2O_2 + R - NH_2 + H^+ = R - NH^3 + HOO^- + H^+ \quad (5)$$

The hydroperoxide anion is very unstable and easily decomposed to high reactive hydroxyl radical (OH•),

$$HOO^- \rightarrow OH^- + O\bullet \quad (6)$$
$$H_2O_2 + HOO^- \rightarrow OH\bullet + O_2\bullet + H_2O \quad (7)$$

The hydroxyl radical is a very powerful oxidant. The main chemical action of HO• with polysaccharide has been demonstrated to be hydrogen abstraction [35]. It reacts with carbohydrates very quickly. Hydroxyl radical pulls off a hydrogen atom and combines with it to form water. During the treatment, the R-$NH_2$ preferentially reacts with $H^+$ to produce R-$NH^{3+}$, which causes the decrease of [$H^+$] and the increase of pH. Besides, $HOO^-$ is rapidly decomposed to HO•, it means that $H_2O_2$ is continually decomposed as shown in Eqs. (5). These radicals undergo further reactions rapidly to form water-soluble oxidation products with low molecular weight. When free radicals are produced rapidly and sufficiently and spread uniformly, free radicals would have easy access to linkages and break the molecular weight of polysaccharides [36]. Following the mechanism, the addition of $H_2O_2$ concentration will be able to produce more hydroxyl radical. The number of hydroxyl radicals produced will reduce molecular weight of κ-carrageenan.

## Functional Properties of Native and Treated κ-carrageenan

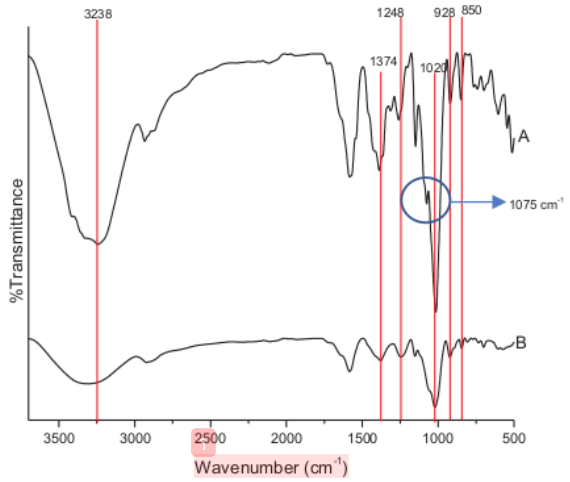


**FIGURE 2**. FTIR spectra of native κ-carrageenan (A) and degraded κ-carrageenan by $H_2O_2$ 1.5% (B).

The structural change of native κ-carrageenan and degraded κ-carrageenan by $H_2O_2$ 1.5% were confirmed by FTIR spectra as shown in Figure 2. The characteristic absorption peaks appearing at 1248 $cm^{-1}$ was attributed to S=O of the sulfate esters and 850 $cm^{-1}$ was C-O-S of the axial secondary sulfate on $C_4$ of galactose, respectively. The peak at 928 $cm^{-1}$ was a characteristic absorption of C-O-C of 3,6-anhydrous-D-galactose. Another peak at 1020 $cm^{-1}$ and 1374 $cm^{-1}$ indicated of glycosidic linkage and sulfates, respectively. The FTIR analysis results show that the functional properties of carrageenan did not change during the oxidation treatment with $H_2O_2$. This is indicated by the presence of sulfate groups in treated carrageenan at wavenumbers 1248, 928 and 850 $cm^{-1}$. It similar to reported by Kalitnik et al. [37].

The compared of the native κ-carrageenan to degraded κ-carrageenan by $H_2O_2$ was described in Figure 2, there was absorption peak lost at degraded κ-carrageenan by $H_2O_2$. It was at wavenymber 1075 $cm^{-1}$ which combination of glycosidic lingkage and C-OH modes. It signified that the degradation process able to break the linkages of κ-

carrageenan. Also, the intensity of the absorption peaks of degraded κ-carrageenan was lower than native κ-carrageenan. It signified that occurred breaks of the linkages in the κ-carrageenan after degraded used $H_2O_2$ concentration 1.5% . As reported by Sun et al. [38], the results of FTIR characterization of degraded κ-carrageenan there was indicated brek of glycosidic lingkage.

## CONCLUSION

κ-carrageenan was effectively degraded by hydrogen peroxide ($H_2O_2$). The extent of the degradation of κ-carrageenan achieved amount 56.5% with operation condition 1.5% $H_2O_2$ concentration, temperature 50 °C and pH 7. The results of the functional properties of the molecular weight of κ-carrageenan used IR spectral analysis (FT-IR) showed that there is no change in the content of the sulfate of κ-carrageenan.


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