Effect of pH on Depolymerization of κ-Carrageenan by Ultrasound, Ozone and Their Combination

A Prasetyaningrum¹, B Jos¹, Y Dharmawan², R Ratnawati¹, S Riyandita¹, and R. Scesario¹

¹Department of Chemical Engineering, Diponegoro University
²Departement of Public Health, Faculty of Public Health, Diponegoro University
Jl. Prof. Soedarto, Kampus Undip Tembalang, Semarang 50275, Indonesia

Corresponding author: ajiprasetyaningrum@gmail.com

Abstract. κ-carrageenan is sulphated linear polysaccharides of D-galactose and 3,6-anhydro-D-galactose extracted from certain red seaweeds of the Rhodophyceae class. They have been extensively used in the food and pharmaceutical industry. However, the use of the κ-carrageenan in the biomedical field is inhibited by its easy to form a gel and a high viscosity. Therefore, it is necessary to production of low molecular weight of carrageenan using depolymerization methods. The objective of this research is to study the effect of pH to reduced the molecular weight of κ-carrageenan with ozonation (O₃), ultrasonication (US) and the combination of O₃ and US (O₃–US) methods. The experimental result shows that the O₃–US treatment can decrease the molecular weight of κ-carrageenan until 80.77% at pH 7 during 20 minutes of treatment. Depolymerization of κ-carrageenan at pH 3 has the highest percentage compared to pH 7 and pH 10. The percentage depolymerization of κ-carrageenan using O₃–US method at pH 3, 7 and 10 are 94.22%; 80.77% and 75.85%, respectively.

1. Introduction
κ-carrageenan are sulphated linear polysaccharides of D-galactose and 3,6-anhydro-D-galactose extracted from certain red seaweeds of the Rhodophyceae class. They have been extensively used in the food industry as thickening, gelling and protein-suspending agents, and more recently by the pharmaceutical industry as excipient in pills and tablets. Besides the well-known biological activities related to inflammatory and immune responses, carrageenan are potent inhibitors of herpes and HPV viruses and there are indications that these polysaccharides may offer some protection against HIV infection [1]. However, the use of the κ-carrageenan in the biomedical field is inhibited by its easy to form a gel and a high viscosity. κ-carrageenan therefore needs to be depolymerized to lose the viscosity to be utilized in the biomedical field. Depolymerization of κ-carrageenan can be done by several methods, such as by using ultrasonification (US). US waves can break down molecules with two main processes, namely acoustic streaming and acoustic cavitation. Acoustic streaming is a sound wave that moved into the liquid, so that it formed a...
movement of liquids with longitudinal wave [2]. Acoustic cavitation begins from the solubility of gases into a liquid called phase formation of bubbles, bubble growth phase, then until the outbreak of the bubble. Research on the depolymerization of carrageenan using US was done by Lii et al. [3]. The research was done by making the solution of κ-carrageenan 1.5% w/v. US radiation under operating conditions 35 Hz, 300 W/cm² and a temperature of 50°C. The result showed that κ-carrageenan depolymerization was shown by the tendency of viscosity decrease of κ-carrageenan solution to the US operation time.

Ozonation reaction is another method that can be used for depolymerization of carrageenan. Seo et al. [4] showed that O₃ was able to decrease intrinsic viscosity in several of O₃ time. This research demonstrated that O₃ treatment of chitosan in acetic acid solution resulted in reduction of molecular size with destruction of chitosan pigments. The procedure of O₃ treatment using microbubbles and transforms ozone gas into small sized bubbles (of sizes less than 10 µm) in water. These small bubbles increase the dissolving potential and expand the oxidizing efficiency of ozone. Therefore the objective of this research is to study the effect of pH to reduce the molecular weight of κ-carrageenan using O₃, US and the combined of O₃–US methods.

2. Material and Methods

2.1. Material

Commercial refined κ-carrageenan was used as material and produced by CV. Karagen Indonesia. The refined κ-carrageenan was used directly without any purification. The raw material used in this work was κ-carrageenan derived from seaweed *Kappaphycus alvarezii*. To produce 1% (w/v) solution, purified κ-carrageenan was completely dissolved in distilled water. The mixture was heated to 70°C and stirred for 15 minutes. The pH of the κ-carrageenan solution was adjusted by adding HCl with 37% of purity (E. Merck Cat. No. 100317) or NaOH with >99% purity (E. Merck Cat. No. 104698). All chemical reagents were of analytical grade and directly used without further purification.

2.2. Ozonation of Carrageenan

A glass reactor equipped with an ozone bubble diffuser was used to carry out the experiments. Ozone gas with a concentration of 80±2 ppm was produced by an ozone generator (Dipo Technology Indonesia). In this research, the production of ozone is generated by passing air between two electrodes with a high potential difference (approximately 30 kV). The ozone was diffused into the solution using micro bubble diffuser at a constant flow rate of 3 L.min⁻¹. The experiments were conducted at pH of 3, 7, and 10. The pH of the κ-carrageenan solution was adjusted with a pH meter (Hanna Instruments HI 98107) and adjusted with hydrochloric acid or sodium hydroxide solution. The ozone treatment was conducted at different times, i.e. 0 (control), 5, 10, 15, and 20 minutes and temperature 29±1°C. Ozone-treated κ-carrageenan was reacted with isopropyl alcohol and the precipitate of oligo κ-carrageenan was filtered with Buchner funnel. For further analysis, the residue was dried at 60°C for 24 h in a forced air oven.

2.3. Ultrasonification Treatment

Two hundred milliliters of sample was brought to specified temperatures (29±1°C) and put into ultrasonic device, Krisbow type KLS 303365, with frequency of 42 kHz. The device was equipped with thermostatic water bath. To minimize error due to uneven power transfer and temperature fluctuation during the ultrasonic process, the sample was placed right above the ultrasound source. The process was run in various times (0, 5, 10, 15, 20 minutes).

2.4. Molecular Weight Determination

The number-average molecular weight of κ-carrageenan was determined by Mark-Houwink equation relating intrinsic viscosity to the molecular weight [5] as follows:

\[ [\eta] = k_{MH} \cdot M^a \] (1)
where $[\eta]$ is intrinsic viscosity, $k_{MH}$ and $\alpha$ are constants, and $M$ is the number-average molecular weight. The values of $k_{MH}$ and $\alpha$ for the $\kappa$-carrageenan solution in water at 25°C are 0.00778 and 0.90, respectively [6, 7]. The intrinsic viscosity is obtained using Huggins equation:

$$\frac{\eta_{sp}}{C} = [\eta] + k_{H}[\eta]^2C$$

(2)

where $\eta_{sp}$, $C$, and $k_{H}$ are specific viscosity, concentration of the solution, and Huggins constant of which value is 0.3 [6]. The percentage of depolymerization of $\kappa$-carrageenan was calculated using the following equation:

$$\text{Depolymerization} (%) = (M_i - M_t) / M_i * 100$$

(3)

where $M_i$ is the initial molecular weight of $\kappa$-carrageenan and $M_t$ is the molecular weight of $\kappa$-carrageenan after $t$ minutes of treatment.

3. Results and Discussion

3.1. Effect of $O_3$, US and combination of $O_3$–US on Depolymerization of $\kappa$-Carrageenan

Based on Figure 1, it can be seen that $O_3$–US combination is very effective in depolymerizing $\kappa$-carrageenan compounds. The combination of US and $O_3$ can decrease the molecular weight of $\kappa$-carrageenan until 80.77% at pH 7. At the similar condition, $O_3$ and US methods only decrease the molecular weight of 75.98% and 56.47%, respectively during 20 minutes of treatment. The similar result reported by Pengphol et al. [8], those combinations of $O_3$–US on degradation of chlorpyrifos was more effective than using individual US and $O_3$ method. Ince et al. [9] observed that the combined of $O_3$–US treatment achieved results twice as fast as $O_3$ alone, while no significant effect was observed in the control experiments with US alone. Oxidation plays a similar role in both of ozonolysis and sonolysis pathways, and some free-radical present during $O_3$ and sonolysis process [10]. The $O_3$–US combination produced air bubbles by the cavitations effect of ultrasound, allowing most ozone to enter the liquid phase or react on the gas or liquid interface. The physicochemical properties of the $O_3$–US combination enhanced the rate of direct reactions and hydroxyl radical’s oxidation of intermediate products [11]. The rapid depolymerization of organic contaminants using $O_3$–US combination, resulted in an increase of hydroxyl radicals [12].

Ozone depolymerization involves free radicals (OH *) to react with organic compounds and break the bonds [13]. Depolymerization reactions in $\kappa$-carrageenan are suspected to occur due to disconnection of the glycosidic bond. This refers to the research of Seung-Wook, et al. [14] that the depolymerization of chitosan by ozone results from the breaking of the glycosidic bond. According to Seung-Wook, et al. [14], the termination of the glycosidic bond is caused by electrophilic breakage occurring in carbon C(1).
The US process is strongly influenced by the power and frequency used during operation. According to the experiments performed by Kidak [15], the enhancement of power and frequency of US was increased the percentage depolymerization of organic compounds.

### 3.2. Effect of pH on Depolymerization of κ-Carrageenan

For all of methods, percent depolymerization of κ-carrageenan at pH 3 has the highest percentage compared to pH 7 and pH 10. This means that depolymerization is more dominated by acid hydrolysis than by the process of O₃ and US itself. Based on Figure 2, the percentage depolymerization of κ-carrageenan using O₃–US method at pH 3, 7, and 10 are 94.22%; 80.77% and 75.85%, respectively.

![Figure 2. Effect of pH on Depolymerization of κ-carrageenan by O₃, US, and Combination of O₃–US](image)

pH is one of the most important parameter to be studied, since it is strongly related to the degree of depolymerization of carrageenan. The similar result reported by Prasetyaningrum et al. [16] that the rate constant value of depolymerization of κ-carrageenan by ozone treatment at lower pH is slightly larger than that at higher pH. The apparent rate constant at pH 3 is 4.3 fold of that at pH 7.

The similar results, Wang et al. [17] reported that the significant reductions of the viscosity of κ-carrageenan at lower pH, due to a disconnection reaction involving acid hydrolysis. Seo et al. [4] also reported a rapid decrease in viscosity at low pH. During 5 minutes of ozonation, the viscosity of chitosan decreased until 91% (from 331 to 29 cP). Klein et al. [18] reported depolymerization of cassava flour through ozonation process at different pH. The ozonation was conducted at 25°C with ozone concentration of 13 mg L⁻¹. The results showed that pH 3.5 was more effective in reducing the viscosity of cassava starch solution.

This suggests that depolymerization of κ-carrageenan involves not only O₃ and US but also involving acid hydrolysis at low pH. This means that the combinations of ozone-ultrasound and low pH give the significant effect of increasing the rate of depolymerization of κ-carrageenan.

### 4. Conclusion

The combined of O₃–US methods to depolymerize κ-carrageenan has been conducted. The results showed that O₃–US treatment and pH have positive effects on the depolymerization. The experimental
result that the combination of $O_3$–US can decrease the viscosity of $\kappa$-carrageenan until 80.77% at pH 7. pH is one of the most important parameter to be studied, since it is strongly related to the degree of depolymerization of $\kappa$-carrageenan. At pH 3, percent depolymerization of $\kappa$-carrageenan has the greatest percentage compared to pH 7 and pH 10.

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References
[1] Campo V L, Kawano D F, Silva Júnior D B, Carvalho I 2009 Carrageenans: biological properties, chemical modifications and structural analysis Carbohydrate Polymers 77: 167-180.
[2] Dolatowski Z J, Stadnik J, Stasiak D 2007 Applications of ultrasound in food technology Acta Sci. Pol., Technol. Aliment 6 (3): 89-99.
[3] Lii C, Chen C, Yeh A, Lai V M 1999 Preliminary study on the degradation kinetics of agarose and carrageenans by ultrasound Food Hydrocolloids 13, 477–481.
[4] Seo S, King M, Priyawiwatkul W 2007 Simultaneous depolymerisation and decoloration of chitosan by ozone treatment Journal of Food Science, 729: C522-C526.
[5] Lai V M-F, Lii C-Y, Hung W-L, Lu T-J 2000 Kinetic compensation effect in depolymerisation of food polysaccharides Food Chemistry, 68: 319-325.
[6] Vreeman H J, Snoeren T H M, Payens T A J 1980 Physicochemical investigation of $\kappa$-carrageenan in the random state Biopolymers, 19: 1357-1354.
[7] Rochas C, Rinaudo M, Landry S 1990 Role of the molecular weight on the mechanical properties of kappa carrageenan gels Carbohydrate Polymers, 12, pp. 255-266.
[8] Pengphol S, Uthaibutra J, Arquero O, Nomura N, Whangchai K 2012 Oxidative degradation and detoxification of chlorpyrifos by ultrasonic and ozone treatments Journal of Agricultural Science 4 (8):164-172.
[9] N Ince, M Stefan, J Bolton, J 1997 Adv.Oxidation Technol 2, 442.
[10] Schramm J D, Hua I 2001 Ultrasonic irradiation of dichlorvos: decomposition mechanism Water Research, 35(3): 665-674.
[11] Cui M, Jang M, Cho S H, Elena D, Khim J 2011 Enhancement in mineralization of a number of natural refractory organic compounds by the combined process of sonolysis and ozonolysis (US/O3) Ultrasoectrics Sonochemistry, 18, 773-780.
[12] Weavers L K, Ling F H, Hoffmann M R 1998 Aromatic compound degradation in water using a combination of sonolysis and ozonolysis Environmental Science & Technology, 32(18), 2727-2733.
[13] Whangchai K, Uthaibutra J, Phiyanalimmat S, Pengphol S, Nomura N 2011 Effect of ozone treatment on the reduction of chlorpyrifos residues in fresh lychee fruits Ozone: Science & Engineering, 33, 232-235.
[14] Seung-Wook S 2006 Depolymerization and Decolorization of Chitosan by Ozonation Treatment Seoul: Chung-Ang University.
[15] Kidak R, Dogan S 2012 OC-11:Degradation of Alachor by Ultrasound, Ozone, and Their Combination (Turkey: Cyprus International University).
[16] Prasetyaningrum A, Ratnawati R, Jos B 2017 Kinetic of oxidative depolymerization of $\kappa$-carrageenan by ozone Bulletin of Chemical Engineering & Catalysis 12(2) 2017.
[17] Wang Y, Hollingsworth R I, Kasper D L 1999 Ozonolytic depolymerization of polysaccharides in aqueous solution Carbohydrate Research 319 pp. 141-147.
[18] Klein B, Vanier N L, Khalid M, Pinto V Z 2014 Ozone oxidation of cassava starch in aqueous solution at different pH Food Chemistry 155 pp. 167-173.