ORIGINAL RESEARCH PAPER

EFFECT OF OZONATION PROCESS ON PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF κ-CARRAGEENAN

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Abstract: κ-Carrageenan is a sulfated galactan extracted from red algae (*Rhodophyceae*) which has many functions. However, nonfood applications of κ-carrageenan have been limited by its superior gelling and viscosity properties. The effect of ozonation on physicochemical and rheological properties of κ-carrageenan solution at different *p*H was investigated. κ-Carrageenan solution was prepared in the ratio of 1:100 (w/v) and was treated with dissolved ozone with concentration of 80±2 ppm. This ozonation was conducted at different times and *p*H. The viscosity of ozone-treated κ-carrageenan solution was analyzed using Brookfield viscometer and the sulfate content was determined using FT-IR spectra and barium chloride-gelatin method. The results show that the viscosity of ozone-treated κ-carrageenan decreases appreciably with time. The highest percentage reduction in viscosity occurs at *p*H 3, followed by *p*H 7 and 10. The FT-IR spectra reveals that the chemical structure of degraded κ-carrageenan, in term of sulfate content, is only slightly affected by the ozone treatment.

Keywords: *depolymerization, κ-carrageenan, ozone treatment, viscosity, sulfate content*

INTRODUCTION

Carrageenan is generic name for a family of polysaccharides, obtained by extraction from certain species of red seaweeds (*Rhodophyta*). Carrageenans are high molecular weight linear polysaccharides comprising repeating galactose and 3,6-anhydrogalactose (3,6 AG) units, both sulfated and non-sulfated, joined by alternating α-(1,3) and β-(1,4) glycosidic links [1 – 3]. In food industry, carrageenans are widely utilized due to their excellent physical functional properties, such as thickening, gelling and stabilizing abilities [4, 5]. Carrageenans have many functions, however their further utilization in nonfood applications has been limited by their superior gelling and viscosity properties. Changing high molecular weight fraction (HMWF) of carrageenans into low molecular weight fraction (LMWF) of carrageenans will increase the bioavailability and expand the potential applications of carrageenans in pharmaceutical and biomedical fields [6–8]. It was reported that LMWF of carrageenans had a significant role in biological activity such as anticoagulant [9 – 12], tumor growth inhibition [13 – 15], antivirus [7, 12, 16, 17], antioxidant [9, 18], and other biological activities. Carrageenans have a high electronegative charge density from their sulfated esters which allow them to interact electrostatically with specific proteins, triggering in consequence their biological effects. LMWF of carrageenan shows stronger antioxidant activity than HMWF of carrageenan, because it can interact with cells more efficiently and effectively [1, 19]. The biological activity of LMWF of carrageenan depends on the size of the molecule, the degree of sulfation, and branching of glycosides [8, 20].
Several different techniques have been used to depolymerize carrageenan, such as thermal depolymerization [21], acid hydrolysis [7, 16, 22 – 25], and enzymatic hydrolysis [13, 14, 16, 23, 26, 27]. Other techniques were by microwave [28], sonication [29 – 31], irradiation [18, 32], and oxidation using H_2O_2 [22, 33].
Ozone is a powerful oxidant with oxidation–reduction potential of 2.07 V [34]. There are many advantages of ozone application, *i.e.*, it does not leave a residue, it can be generated on site, it requires no storage and subsequent disposal of chemical containers, and it is recommended by Food and Drug Administration (FDA) of the United State [35, 36]. Several recent studies have demonstrated the potential of ozone in waste treatment process and depolymerization of macromolecular polysaccharides such depolymerization of wheat starch, chitosan and cassava starch [36 – 38]. Nevertheless, up to the moment, study on depolymerization of carrageenan through ozonation process has not been reported. The development of an emerging technology for depolymerization of HMWF of carrageenan to LMWF of carrageenan, without modification of its chemical structure, is of great interest. This study aims to determine the effect of ozonation process on physicochemical and rheological properties of κ-carrageenan solution at different *p*H.

MATERIALS AND METHODS

Raw materials

Semi refined κ-carrageenan with average molecular weight (MW) of 545 kDa was obtained from CV. Karagen Indonesia (Central Java, Indonesia). The *p*H of

κ-carrageenan solution was adjusted by adding HCl (E. Merck Cat. No. 100317) or NaOH (E. Merck Cat. No. 104698).

Ozonation treatment

κ-carrageenan (1 : 100, w/v) was dissolved in distilled water at 70 °C. The experiments were carried out in a glass reactor equipped with an ozone bubble diffuser. Ozone was generated using ozone generator (Dipo Technology Indonesia) with concentration of dissolved ozone of 80 ± 2 ppm. Ozone was bubbled into the solution with a constant flow rate of 3 L·min^{-1}. The ozone treatment was conducted at different times, *i.e.*, 0 (control), 5, 10, 15, and 20 minutes. The experiments were conducted at *p*H 3, 7 and 10 and constant stirring speed (200 rpm). Ozone-treated κ-carrageenan was collected, rinsed with distilled water, and filtered with buchner funnel. The residue was dried at 60 °C for 24 h in a forced air oven.

Viscosity analysis

The viscosity of carrageenan in aqueous 0.1 M NaCl solution was measured using a Brookfield viscometer of RDVE model (Brookfield Engineering Laboratories, Stoughton, MA). The solution was put in the sample container placed in water bath to equilibrate the temperature to 65 ± 0.1 °C. Viscosity was measured using spindle number 2 and the speed of the spindle was set at 60 rpm. Viscosity value was read in units of centipoise (cP). The measurement was done in triplicate. The percentage of decrease in viscosity was calculated using the following equation:

$$\text{Decrease in viscosity (\%)} = [(V_i - V_t) / V_i] \times 100$$

where V_i is the initial viscosity and V_t is the viscosity after t minutes of ozonation.

Spectral analysis

FT-IR spectra of native and ozone–oxidized of κ-carrageenan samples were measured using an FT-IR Prestige-21, Shimadzu, in wavenumber range of 4000–400 cm^{-1}. Pellets were prepared by mixing the sample with KBr with a ratio of 1 : 100 (sample : KBr).

Sulfate analysis

The sulfate contents of both native and ozone-treated κ-carrageenan were determined using barium chloride-gelatin method [39]. A known amount of sample (W_1 g) was hydrolyzed using 50 mL of 1 N HCl by heating at boiling temperature for 30 minutes. Ten milliliters of 0.25 M BaCl$_2$ solution was added to the reaction mixture. The mixture was then cooled to room temperature and kept for 5 hours. BaSO$_4$ precipitate was filtered with ashless filter paper and incinirated in muffle furnace at 700 °C for 1 hour. The ash was weighed as W_2 g and the content of sulfate can be calculated according the equation:

$$\text{\% Sulfate} = (W_2 / W_1) \times 41.16$$

RESULTS AND DISCUSSION

Effect of ozonation on viscosity of κ-carrageenan solution

The effect of ozone treatment on the viscosity of *κ*-carrageenan solution is shown in Table 1. The viscosity rapidly decreases in the first 15 minutes and then slowly decreases afterwards. The results show that ozonation process gives significant effect on the reduction of κ-carragenan viscosity. In 15 minutes of ozonation, the viscosity decreases from 45.13±0.12 cP (control) to 28.73±0.15 cP, 25.17±0.25 cP, 12.73±0.21 cP at *p*H 10, 7, and 3, respectively. The reduction of viscosity of κ-carragenan solution due to ozone treatments is closely related to the effectiveness of depolymerization process.

Table 1. *Viscosity of κ-carrageenan solution after ozone treatment at different pH*

Time	Viscosity [cP]		
	*p*H 10	*p*H 7	*p*H 3
5	34.70±0.26	32.73±0.41	26.63±0.32
10	31.87±0.38	28.53±0.32	18.53±0.15
15	28.73±0.15	25.17±0.25	12.73±0.21
20	28.23±0.31	24.83±0.30	11.17±0.23

The results of this study show the same trend as chitosan ozonation reported by No et al. [40]. Their study showed that ozone treatment considerably decreased the viscosity by 63 % (206 cP) after 10 min of treatment, compared to the untreated chitosan solution (556 cP). Chan et al. [41] observed a significant decrease in the viscosity of 5 % (w/w) ozone-oxidized corn, sago, and tapioca starches solutions at 25 °C compared to unmodified starch. Tiwari et al. [42] found that treatment with 2.4 - 7.8 % (w/w) ozone for 5 – 10 minutes affected the properties of guar, CMC, and pectin. Cataldo et al. [43] observed only a 5 % change in viscosity of gelatin after 3 hours of ozonation with 450 mg of ozone bubbled into a solution of gelatin.

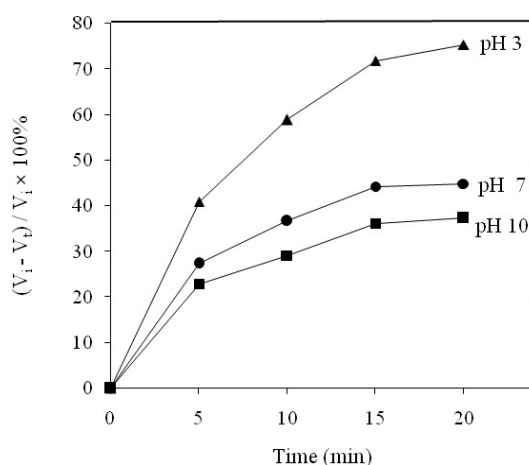The effect of *p*H on the decrease of κ-carrageenan solution viscosity is presented in Figure 1.

Figure 1. *The decrease of κ-carrageenan viscosity (%) at different pH*

The highest percentage of reduction of κ-carrageenan solution viscosity occurs at pH 3. This study shows that in 15 minute, the viscosity changes rapidly. At pH 3 the viscosity reduces by 71.7 %, followed by pH 7 and 10 with viscosity reduction of 44.2 % and 36.2 %, respectively.

Seo et al. [37] also reported a rapid decrease in viscosity at low pH. The viscosity of chitosan decreased by 91 % (from 331 to 29 cP) in initial 5 minutes of ozonation. Klein et al. [38] 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.

Several studies suggest that pH influences the degradation of macromolecules. Wang et al. [44] who investigated the depolymerization of polysaccharide by ozone treatment, stated that carbohydrates degraded by ozone in aqueous solution by several mechanisms: ozonolytic degradation of $β$-D-glycosidic linkages, oxidative degradation by radical species (hydroxyl radicals), and acid hydrolysis. Ozonolytic oxidation leads to selective depolymerization of polysaccharides, whereas radical reactions and pH-induced acid hydrolysis result in non-selective degradation pathways. The decrease in viscosity for oxidized polysaccharide can be attributed to the partial cleavage of glycosidic linkages, resulting in a decrease of molecular weight [37, 38, 44]. According to Seo et al. [37] depolymerization of a polysaccaharide is mainly due to the oxidative destruction of $β$-D-glucoside bonds between units by the electrophilic attack on the C(1)-H bond by ozone molecules.

FT-IR spectroscopy analysis

The FT-IR spectra can provide useful information of polymeric hydrogen bond network. Usually, carbohydrates show high absorbances in the region of 1270 – 600 cm^{-1}, that is within the so-called "fingerprint region" [45, 46]. The position and intensity of the bands are specific for every polysaccharide, allowing its possible identification. The structure of the various types of carrageenans is defined by the number and position of sulfate groups, the presence of 3,6-anhydro-D-galactose, and conformation of the pyranosidic ring [6]. The differences on chemical composition and configuration are responsible for their interesting rheological properties which foster many applications they have, mainly as gelling, thickening and stabilizing agents, in the food, pharmaceutical, and cosmetics industries.

The FT-IR spectra for native and ozone-treated κ-carrageenan are presented in Figure 2. The existence of sulfate groups in the FT-IR spectra of κ-carrageenan can be identified in the region of 1230-1270 cm^{-1}. A clear band at 840-850 cm^{-1} indicates C_4 position (in the galactose ring). The band at 928-933 cm^{-1} is associated with a coupling of the C–O stretching vibrations of 3,6-anhydro bridges [45, 46]. This research shows that absorption peaks appearing at 1257 and 845 cm^{-1} contribute to S=O of sulfate esters and C–O–S of axial secondary sulfate on C–4 of galactose, respectively. Meanwhile, the band at 930 cm^{-1} is characteristic of C–O of 3,6-anhydro-D-galactose. These characteristic absorption peaks found in the FT-IR spectra are indicative of the structural integrity of κ-carrageenan in the depolymerization process. This research shows the existence of sulfate group in the κ-carrageenan even after the ozonation process.
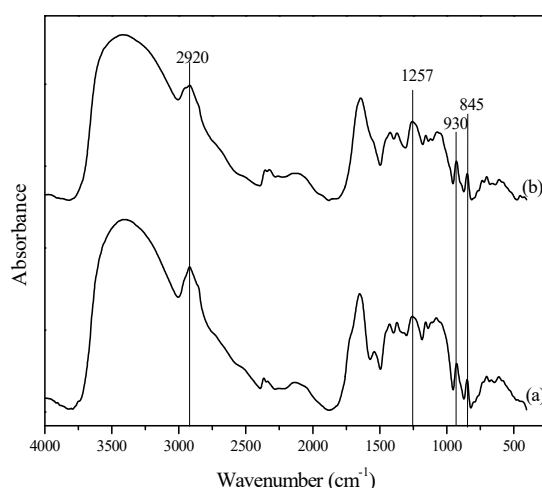
Figure 2. FT-IR spectra of κ-carrageenan (a) Native (b) Ozone treatment at pH 3

Several researches suggest that the biological activity of LMWF of carrageenan depends on the presence of sulfate groups. It is very important to determine the amount of sulfate in the LMWF of κ-carrageenan after ozonation process. Common methods for determination of sulfate content are by gravimetry, spectrophotometry or turbidimetry. However, these methods are destructive and not able to indicate the location of the sulfate group in the carrageenans.

Infrared spectroscopy (IR) can also be used to determine the sulfate content in the sample. The IR technique is rapid and non destructive and it requires only a few mg of sample. The quantitative methods for determining functional groups content in carrageenans is by calculating the absorbance ratios: 1257/2920 for total sulfate, 845/2920 for galactose-4-sulfate, 805/2920 for 3,6-anhydrogalactose-2-sulfate, and 930/2920 for 3,6 anhydrogalactose [46]. The absorbance at 2920 cm^{-1} which is related to C–H is used as an index for total polysaccharide content. The absorbance of bands in the infrared spectrum is calculated using equation: $A = \log T_b/T_p$. Symbols refer to transmittance (T) and absorbance (A) of base line (b) and peak (p).

The ratio of 1257/2920 cm^{-1} was calculated to represent the amount of total sulfate in native and ozone-oxidized κ-carrageenan. After 20 minute of ozonation, the absorbance ratio of 1257/2920 cm^{-1} for ozone-oxidized κ-carrageenan at *p*H 10, 7, and 3 are 0.23, 0.22 and 0.21, respectively, in comparison with the native κ-carrageenan (0.25). The remaining sulfate in the ozone-treated carrageenan can be calculated using this ratio. It is found that the sulfate remains approximately 92 %, 88 % and 84 % at *p*H of 10, 7, and 3, respectively. FT-IR results clearly show that there are no significant changes in the functional groups and chemical structure of κ-carrageenan after ozone treatment.

Effect of ozonation on sulfate content of κ-carrageenan

As shown in Figure 3, the sulfate content after the ozonation reaction does not significantly decrease. The sulfate content of native carrageenan is 13.37±0.12 %. After 20 minutes of ozone treatment at *p*H 10, 7, and 3, the sulfate contents are reduced to 12.14±0.25 %, 11.93±0.19 %, and 11.52±0.27 %, respectively. This finding suggests that the sulfate groups are only slightly removed during ozonation process. The remaining sulfate contents in κ- carrageenan after 20 minute of ozonation at *p*H of 10, 7,

and 3, compared to that of native κ-carrageenan, are approximately 91 %, 89 %, and 86 %, respectively, of the initial contents.
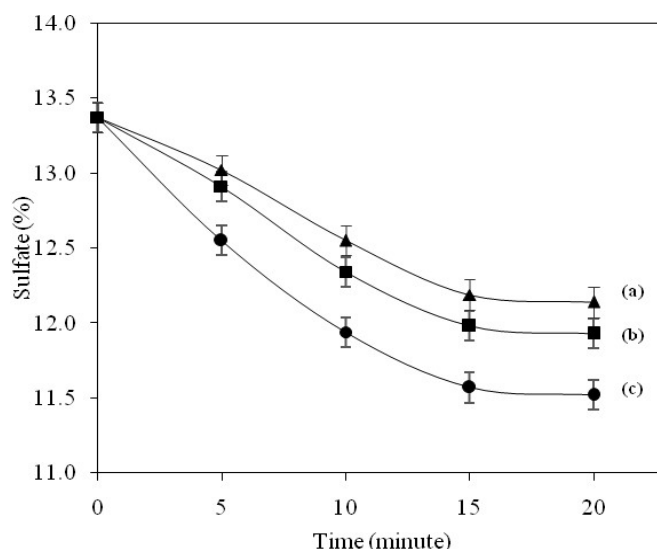

Figure 3. *Sulfate content in κ-carrageenan after ozonation*
(a) at pH 10 (b) at pH 7 (c) at pH 3

Some other groups of researchers reported similar results to this work where depolymerization gives no effect on carrageenan sulfate content [7, 16, 32]. The sulfate remains, after irradiation, approximately 90 %, 83 % and 71 % for κ-, ι-, and λ-carrageenan, respectively [32]. κ-Carrageenan is the most stable to desulfation during acid depolymerization. Sun et al. [22] reported a comparative investigation of the remaining sulfate content in κ-carrageenan after H_2O_2 depolymerization, enzymatic digestion, partial reductive hydrolysis and HCl hydrolysis. With initial content of sulfate of 15.66 ± 0.30 %, the sulfate content after H_2O_2 depolymerization, enzymatic digestion, partial reductive hydrolysis, and HCl hydrolysis, were 15.47 ± 0.43 %, 15.27 ± 0.35 %, 15.55 ± 0.16 %, and 11.87 ± 0.51 %, respectively. Karlsson et al. [47] also reported that under mild acidic hydrolysis (over at least 2 h), κ-carrageenan did not desulfate and did not change their hydrodynamic volume as a result of loss of charge. In this work, the sulfate content determined using FT-IR and barium chloride-gelatin methods show similar results. It clearly indicates that there is no significant loss of sulfate upon ozonation of κ-carrageenan.

CONCLUSIONS

The effect of ozonation process on κ-carrageenan's physical and rheological properties has been investigated. The ozonation process gives significant effect on the reduction of κ-carragenan solution viscosity. The viscosity rapidly decreases in the first 15 minutes of ozonation. The highest percentage of reduction of κ-carrageenan solution viscosity occurs at *p*H 3. The FT-IR spectra shows that there are no significant changes in the functional groups of κ-carrageenan after ozone treatment. The remaining sulfate contents in κ-carrageenan after 20 minute of ozonation at *p*H of 10, 7, and 3, compared

to that of native κ-carrageenan, are approximately 91 %, 89 %, and 86 %, respectively, of the initial contents. This finding suggests that the sulfate groups are only slightly removed during ozonation process.

ACKNOWLEDGMENT

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