

Chemical and Spectral Characterization of The Ozonation Products of κ -Carrageenan

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Abstract. Kappa (κ -) carrageenan oligomers are known to have several biological activities. Recent progress in the development of modified κ -carrageenan has resulted low molecular of κ -carrageenan. Ozone is a powerful oxidant and considered for depolymerization of κ -carrageenan. However, few studies have investigated the changes in κ -carrageenan properties associated with ozone treatment. This study would investigate on the changes in chemical structure after ozonation process. The experiments were carried out in a glass reactor equipped with an ozone bubble diffuser. Ozone with concentration of 80 ± 2 was bubbled into the solution. The ozone treatment was conducted at different times, i.e., 0 (control), 5, 10, 15, and 20 minutes. The experiments were conducted at pH 7 and constant stirring speed (200 rpm). Ozone-treated κ -carrageenan was dried at 60°C for 24 h in a forced air oven. The chemical and spectral analyses of κ -carrageenan after ozonation process were carried out using UV-Vis and FT-IR spectroscopy. These changes are seen in the UV spectra as a high intensity of absorbance peak at 290 nm. It is shows that ozonation of κ -carrageenan leads to some chemical changes such as the formation of carbonyl, carboxyl or double bonds. 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: κ -carrageenan, ozonation, spectral characterization

1 Introduction

κ -Carrageenan is a considerable gelling sulfated polysaccharide extracted from *Kappaphycus alvarezii* (Rhodophyceae). It comprises of repeating disaccharide units of alternating (1/3)- α -D-galactose-4 sulphate, (1/4)- β -3 and 6-anhydro-D-galactose residues [1-4]. The size or degree of polymerization of κ -carrageenan plays a critical role in determining their application. Large molecular size of κ -carrageenan have limited applications as it is insoluble in water and it forms viscous solution. Converting the High Molecular Weight Fraction of carrageenan (HMWF) into Low Molecular Weight of carrageenan (LMWF) will construct the bioavailability and enlarge the potential uses of carrageenan in pharmaceutical and biomedical [5-21].

Currently, LMWF of carrageenan has been produced by several different techniques, such as thermal

depolymerization [20], acid hydrolysis [6, 16, 21 - 25], enzymatic hydrolysis [12, 13, 15, 26, 27], microwave-assisted depolymerization [28], sonication [29 - 31], irradiation [17, 32], and oxidation using H_2O_2 [21, 33]. To produce a series of the carrageenan oligomers, acidic hydrolysis has been considered as a common and rapid method. However, the use of chemicals causes an increasing of the environmental pollution level. The enzymatic method, either using specific or non-specific enzymes, is a relatively not recommended. The enzymatic method need *kappa carragenase* enzyme that relatively high-priced and complex process [27]. Due to the high oxidation potential, ozone can be an alternative method to attaining degradation of organic and anorganic compounds [34 - 53].

Polysaccharide depolymerization with ozonation process is widely developed because the ozonation

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method provides a convenient, inexpensive, and easily controllable for producing low molecular of polysaccharide [27]. Ozone has been proven to be able to degrade macromolecules. Ozone has been used to depolymerize for many polysaccharides such as starch [36], chitosan [37], gelatin [43] and cellulose [44].

During ozonation process, may occur the change physicochemical and rheological process. It has been known that depolymerization of polysaccharides by ozonation process leads to some chemical changes such as the formation of carbonyl, carboxyl or double bonds [6, 10, 15]. In the degradation of chitosan, oxidation of functional groups by hydrogen peroxide give the results of ring-opening reaction, the formation of carboxyl groups and deamination [27]. There was limited reports of carrageenan depolymerization treatment by ozonation. The chemical changes which occur in polysaccharides are similar to those with simple carbohydrates. For this reason, our study was to analyze the effect of ozonation for chemical changes of κ -carrageenan.

2 Material and Methods

2.1. Materials

Refined κ -carrageenan was acquired from CV. Karaindo, Center of Java, Indonesia. κ -carrageenan was diluted in hot distilled water (70°C). Carrageenan solution was filtered for separate with the impurities. Purified κ -carrageenan was obtained by precipitation with isopropyl alcohol and dried at 60°C for 24 hours.

2.2 Methods

Water distillation was used to dissolve the purified carrageenan. Purified κ -carrageenan was completely dissolved in distilled water to form 1 % (w/v) solution. The ozonation was carried out at pH 7 and 29 ± 1 °C. The sample inserted glass reactor that equipped with an ozone bubble diffuser. The sample was then ozonated with different time. Ozone gas with concentration of 80±2 ppm was produced by an ozone generator (*dielectric barrier discharge*). Ozone is generated by passing air between two electrodes with 30 kV high potential. Ozone was bubbled into the solution with a constant flow rate of 3 L min⁻¹. This experiments were stirred with constant speed (200 rpm). The pH of κ -carrageenan solution was adjusted with HCL and NaOH. The measuring of pH during ozonation process using pH meter (Hanna Instruments HI 98107). The ozone treatment was carried out at different times, i.e., 0 (control), 5, 10, 15, and 20 minutes. Ozone-treated κ -carrageenan was collected, rinsed with distilled water, and filtered with Buchner funnel. The temperature used to dry the residue is at 60°C for 24 h in a forced air oven for UV-VIS and FT-IR analysis.

2.3 UV-Vis Analysis

The spectra analysis using Shimadzu spectrophotometer. The specification was UV-265 FW and wavelength range (200 - 600 nm). The perform UV-Visible spectroscopy of carrageenan solutions at ambient temperature and at 0.025% (w/v) concentration.

2.4 FT-IR Spectroscopy

The measurement of FT-IR spectra of native and ozone-oxidized of κ -carrageenan samples was using an FT-IR Prestige-21, Shimadzu, in the wavenumber range of 4000–400 cm⁻¹. Pellets were prepared by mixing the sample with KBr. The ratio used is 1: 100 (Sample: KBr).

3 Results and Discussion

The chemical changes that occur in sulfated galactan polysaccharides are the same as those simple carbohydrates. Reducing groups (carbonyl) and acid groups appear and carbon-carbon scission occurs. The chemical changes of κ -carrageenan before and after ozonation process were analyzed with UV-Vis and FT-IR spectra.

3.1. UV-Vis Spectra

Figures 1 show that the peak intensity increases with increasing of ozonation time. The absorbance peak of κ -carrageenan at around 290 nm after ozonation process (5, 10, 15 and 20) minutes ozonation were (0.637; 0.642; 0.774 and 0.777), respectively. For comparison, the absorbance peak of κ -carrageenan before ozonation was 0.587. The intensity of absorbance was increase 32.36 % during 20 minutes of ozonation.

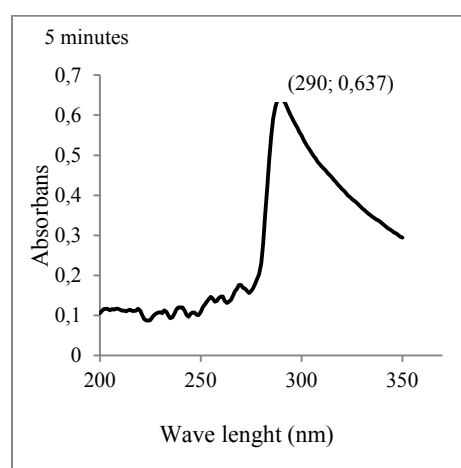


Fig. 1. UV-Vis spectra of 5 minutes ozonated κ -carrageenan

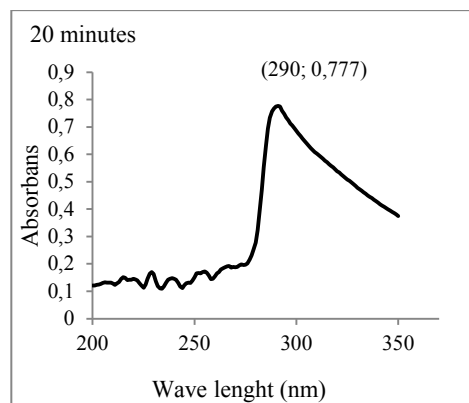


Fig. 2. UV-Vis spectra of 20 minutes ozonated κ -carrageenan

3.2. FT-IR Spectra

The sulfate groups contained in κ -carrageenan before and after ozonation process was identified by FT-IR spectra. Sulfate group in κ -carrageenan recognized in the area of $1230\text{-}1270\text{ cm}^{-1}$. The band at $840\text{-}850\text{ cm}^{-1}$ shows C4 position (within the galactose ring). The band at $928\text{-}933\text{ cm}^{-1}$ is connected with a coupling of the C–O stretching vibrations of 3,6-anhydro bridges [45, 46]. This study shows that absorption peaks 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.

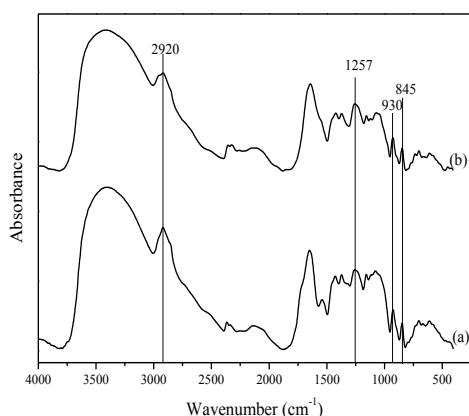


Fig. 3. FT-IR spectra of (a) native and (b) ozonated κ -carrageenan

Meanwhile, the band at 930 cm^{-1} is characteristic of C–O of 3,6-anhydro-D-galactose. FT-IR spectra are indicative of the structural integrity of κ -carrageenan in the process of depolymerization. The biological activity of LMWF of carrageenan relies upon the presence of sulfate groups. This study indicates that even after the process of ozonation, there is sulfate group existence in the κ -carrageenan.

Conclusions

Chemical and spectral characterization of κ -carrageenan during ozonation process has been investigated. The ozonation process gives no significant effect on the

reduction of sulfated content of κ -carrageenan. The UV-Vis spectra shows that the formation of double bonds increase after 20 minutes of ozonation. The FT-IR spectra shows that there are no enormous changes in the functional groups of κ -carrageenan after ozone treatment. This finding suggests that the sulfate groups are only slightly removed during ozonation process.

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