

The Impact of Combination of Ozonation and Ultrasonication Process on Morphological and Chemical Properties of κ -Carrageenan

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Abstract

Carrageenan application in the biomedical field is influenced by the size of molecular weight. Depolymerization process is needed for changing the high molecular weight (HMW) fraction of κ -carrageenan into low molecular weight fraction (LMW) of κ -carrageenan. Ozone is a powerful oxidant and considered for depolymerization of κ -carrageenan. This research was conducted as an investigation on the influence of the combination of ozonation and ultrasonication (OZ/US) on the chemical and morphological characteristics of κ -carrageenan. FTIR and Scanning electron microscopy (SEM) was used to determine the change of chemical and morphological characteristics of κ -carrageenan. The result of this study showed that there was no change of chemical properties of κ -carrageenan during OZ/US treatment. The sulfate content was obtained after OZ/US treatment tends to be stable. Nevertheless, the result of SEM analysis showed that there were changes on morphological characteristics of κ -carrageenan after OZ/US treatment.

Keywords: ultrasonication, κ -carrageenan, ozonation, morphological, chemical

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INTRODUCTION

κ -Carrageenan is extracted from seaweed or red algae (*Rhodophyceae*) which formed from repeated bonds of disaccharide of 1,3-linked β -D-galactose 4-sulfate and 1,4-linked 3,6- anhydro- α -D-galactose (Necas & Bartosikova, 2013; Campo *et al.*, 2009). The size or degree of polymerization of κ -carrageenan plays a critical role in determining their application. LMW of κ -carrageenan have the function for biomedical and pharmaceutical field. The

changing of the HMW of κ -carrageenan into LMW of κ - will increase the bioavailability (Bixler & Porse, 2010).

LMW of κ -carrageenan has been produced by several different techniques. There are thermal depolymerization (Qiet *et al.*, 2005), acid hydrolysis (Prajapati *et al.*, 2014), enzymatic hydrolysis (Yao *et al.*, 2014), microwave assisted depolymerization (Zhou *et al.*, 2006), sonication (Ratnawati *et al.*, 2016; Taghizadeh & Abdollahi, 2015) irradiation (Chiu *et*

al., 2012) and oxidation using H₂O₂ (Lai *et al.*, 2010). 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 increase of the environmental pollution level (Ratnawati & Indriyani, 2018). The enzymatic method, either using specific or non-specific enzymes, is relatively not recommended. The enzymatic method needs kappa carrageenase enzyme that relatively high-priced and complex process (Duan *et al.*, 2016). Due to the high oxidation potential, ozone can be an alternative method to attaining degradation of organic and inorganic compounds (Loures *et al.*, 2013).

Depolymerization of polysaccharide with the combination of OZ/US process is widely developed because of the ozonation method provides an inconvenient and expensive to producing low molecular of polysaccharide (Yamada *et al.*, 2000). Ozone has been used to depolymerization of many polysaccharides such as starch (Sandhu *et al.*, 2012), chitosan (Seo *et al.*, 2007), gelatin (Cataldo, 2007) and cellulose (Wang *et al.*, 1999). Ultrasonication process is simple, cheap, energy-efficient, and suitable for food processing (Akyüz *et al.*, 2008). The driving mechanism for the effects of ultrasound (US) is the phenomena of cavitation, which can be described as the formation and activity of gaseous bubbles or vaporous cavities in a liquid. Ultrasound can be used as a technique for depolymerization. When ultrasound wave is applied to a dilute polymer solution, it generates pressure wave causing cavitation which consists of the nucleation, growth, and collapse of microbubbles. A segment of a polymer molecule in the vicinity of a collapsing bubble moves with much higher velocity than other segments located farther. The velocity gradient causes the polymer chain to stretch and eventually a cleavage at the midpoint of the molecular chain could happen (Akyüz *et al.*, 2008; Caruso *et al.*, 2009). The combination of ozone and ultrasound expected to improve the efficiency of κ -carrageenan depolymerization. There is a limited report of carrageenan depolymerization treatment by OZ/US. The purpose of this study was to investigate the effect of OZ/US for morphological and chemical properties of κ -carrageenan.

MATERIALS AND METHODS

Materials

Purified K-carrageenan obtained from CV. Karaindo, Central Java, Indonesia. Hot distilled water (70°C) is used to dissolve k-carrageenan. Pure carrageenan is obtained by dissolving semi-refined carrageenan and precipitating with isopropyl alcohol. These deposits are filtered and dried at 60°C for 24 hours.

Methods

The κ -carrageenan sample was made by dissolving pure κ -carrageenan (1% b/v). The OZ / US combination process is carried out with pH and

temperature control (pH 7 and temperature $29 \pm 1^\circ\text{C}$). A 200 ml glass beaker equipped with an ozone bubble diffuser is used for depolymerization reactions. The ultrasound process uses an ultrasonic device, the Krisbow KLS 303365 type with a frequency of 42 kHz. The ozonation reaction uses ozone gas produced by ozone generator with a constant flow rate of 3 L min⁻¹ and a concentration of 80 ± 2 ppm. The pH of κ -carrageenan solution was adjusted with HCl and NaOH. The measuring of pH during the ozonation process using pH meter (Hanna Instruments HI 98107). OZ/US treatment are treated for 15 minutes. OZ/US-treated κ -carrageenan was collected and filtered with Buchner funnel. Then the sample is dried with a hair dryer for 3 hours for SEM and FT-IR analysis.

SEM Analysis

Scanning Electron Microscopy (FEI type S 50) is used to test surface morphology and pore size. In short, the sample is fixed on the carbon band. Before being examined, the sample was dried under vacuum and coated with platinum.

FT-IR Spectroscopy

Chemical properties of κ -carrageenan were analyzed by FT-IR Prestige-21Shimadzu. A sample of 0.02 g was mixed with KBr and pressed to form a thin film with ratio 1:100. The spectrum is carried out in the range 4000-400 cm⁻¹.

RESULTS AND DISCUSSIONS

Scanning Electron Microscopy

The results of the analysis of morphological properties of the native carrageenan samples and carrageenan after OZ/US treatment is presented in the Fig. 1. The analysis of Scanning Electron Microscopy (SEM) performed on pH 7 and temperature $29 \pm 1^\circ\text{C}$ during 15 min of treatment. The image of SEM analysis with magnification of 5.000x are shown in Fig. 1.

The SEM micrograph of the κ -carrageenan before treatment shows a uniform and smooth surface. Comparison of the SEM images showed that the surface of κ -carrageenan was relatively brittle and porous after process OZ/US. This is due to the depolymerization process where anhydro galactose and galactose 4-sulfate groups are cut off on the k-carrageenan molecule so that the surface tends to be more coarse and porous (Fojas *et al.*, 2013).

FT-IR Spectra

The results of this research on the effect of the ozonation and ultrasonication process on functional groups of carrageenan is presented in Fig. 2. Infrared spectroscopy is commonly used to determine the chemical structures of polymers. The FTIR spectra obtained were used to identify the presence of functional groups of the chemical structure of polysaccharides (Fojas *et al.*, 2013).

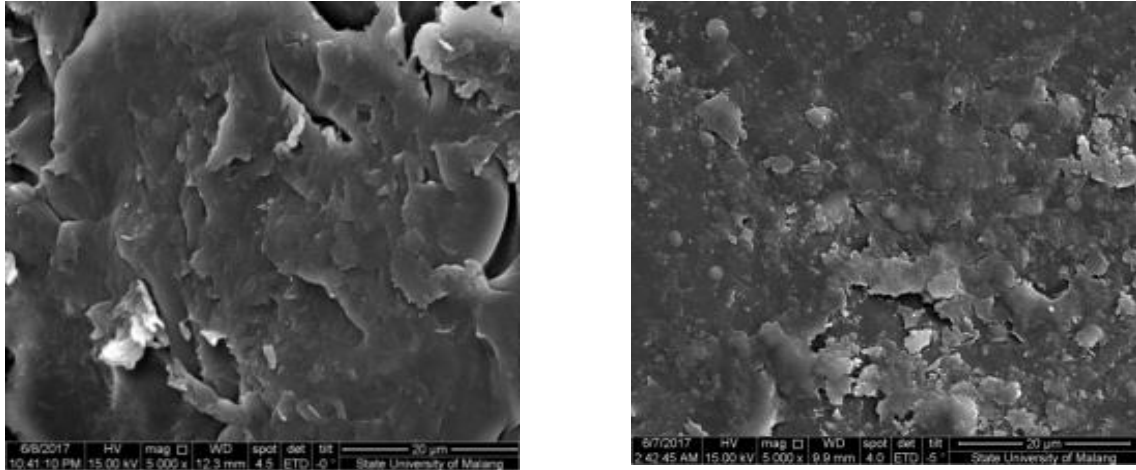


Figure 1. SEM of (a) native and (b) κ -carrageenan after treatment

The FTIR spectra of native and κ -carrageenan after treatment is shown in Fig. 2.

The spectrum of FTIR shows the structural and chemical properties of κ -carrageenan during the process of depolymerization. The FTIR spectra show a broad absorption peak at 3444 cm^{-1} , indicating the OH stretching mode (Liew *et al.*, 2017). The results of FTIR analysis showed that the κ -carrageenan characteristic band occurred at 1248 cm^{-1} and 850 cm^{-1} , which showed the O=S bond = vibration stretching mode and O-SO₃ stretch vibration mode at position C-4 galactose, respectively (Liew *et al.*, 2017). The peak at 928 cm^{-1} represents a coupling of the C-O stretching vibrations of 3,6-anhydro-D-galactose (Mobarak *et al.*, 2012). A characteristic peak at 2931 cm^{-1} corresponds to the C-H stretching vibrations of alkane groups. Another peak observed at 1643 cm^{-1} corresponds to the carbonyl group stretching (Tanusorn *et al.*, 2018). The region around $800\text{--}850\text{ cm}^{-1}$ is used to identify the position of the sulfate group in

carrageenan. There was a difference in the results of the FTIR analysis in the wave number 1012 cm^{-1} , which showed the breaking of the glycosidic bonds in the carrageenan after treatment.

The functional of low molecular carrageenan for biomedical application depends on the presence of sulfate groups (Pomin, 2010). The FTIR spectrum analysis that identifies the sulfate content in carrageenan are in the wavelength range 1248 cm^{-1} and 850 cm^{-1} .

During ozonation and ultrasonication process, the sulfate content peaks therefore no change in the functional groups of κ -carrageenan. This study indicates that after the combine OZ/US treatment, the sulfate content of κ -carrageenan tends not to change. Prajapat and Gogate (2015) reported that no significant chemical structure changes were obtained due to the guar gum after OZ/US treatment.

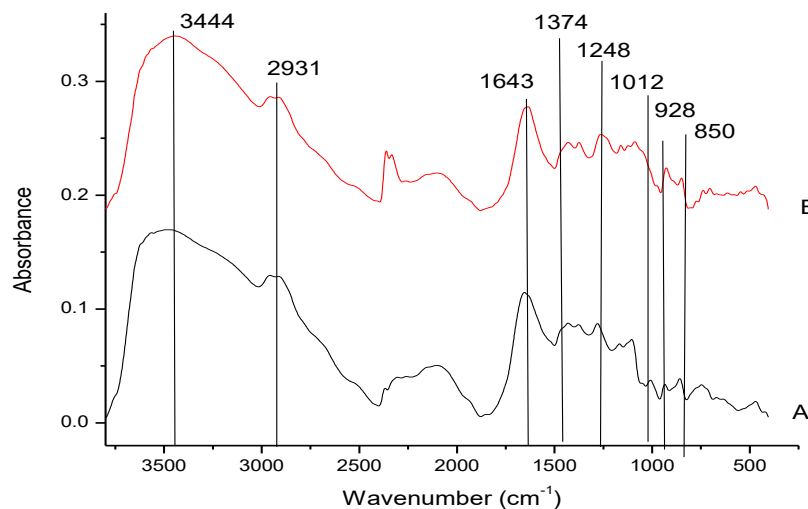


Figure 2. FT-IR spectra of (a) native and (b) κ -carrageenan after treatment

CONCLUSIONS

Morphological and chemical properties of κ -carrageenan after OZ/US process has been investigated. The morphological structure of the samples using SEM showed that the surface of κ -carrageenan was relatively brittle and porous after process OZ/US. This is due to the depolymerization process where anhydro galactose and galactose 4-sulfate groups are cut off on the κ -carrageenan molecule so that the surface tends to be more coarse and porous. The FTIR spectra result clearly shows that there are no significant changes in the functional groups of κ -carrageenan after ozone and ultrasound treatment. This research shows that the sulfate groups are relatively stable during OZ/US treatment.

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