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A COMBINED ULTRASOUND AND OZONE (US/O_3) TREATMENT ENHANCING k-CARRAGEENAN DEPOLYMERIZATION

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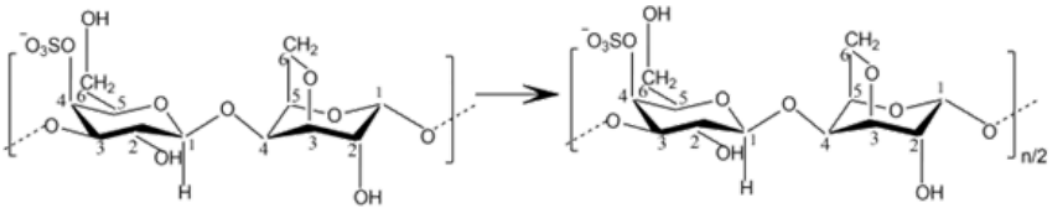
ABSTRACT

INTRODUCTION

Carrageenan is a natural polysaccharide sulfated galactan composed of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose or 4-linked 3,6-anhydro-α-D-galactopyranose [1]. Carrageenans are classified according to the number and position of the sulfate groups. The commercial κ-carrageenan or the high molecular weight fractions (HMWF) of κ-carrageenan have an average molecular weight of 100 kDa - 1000 kDa [1]. The depolymerization process may change HMWF of κ-carrageenan to low molecular weight fractions (LMWF). The latter have biological activities, such as antiviral [2 - 6], anticoagulant [7, 8], antitumor [9, 10] and antioxidant [11, 12] one. The LMWF of κ-carrageenan can penetrate the human cells more effectively when compared with HMWF [2]. The LMWF of carrageenan of a molecular weight size of less than 20 kDa are used for biomedical applications [12]. The changes of the molecular structure of κ-carrageenan from HMWF to LMWF is presented in Fig. 1.

Thermal depolymerization [13], acid-catalyzed hydrolysis [9, 14], enzymatic hydrolysis [10], irradiation [12, 15], and sonication [16, 17] are widely used as depolymerization techniques. The acidic hydrolysis is a common fast method which leads to an increase of the environmental pollution level [9, 14]. The enzymatic method is not preferable because of the relatively expensive and complex process used [10].



HMWF of κ-carrageenan LMWF of κ-carrageenan

Fig. 1. Depolymerization of κ-carrageenan.

Due to its high oxidation potential, ozone can be alternatively used to degrade organic and inorganic compounds [18, 19]. It is well known as a powerful oxidizing agent [18] removing pollutants from the water bodies [20] and depolymerizing polysaccharides. Ozone is generally recognized as safe (GRAS) for use in industrial food preparation by the United States of Food and Drug Administration. However, the application of ozonation has been limited by the low ozone mass transfer rate. The latter may be increased by an ultrasonication treatment [20], which in correspondence with the green technology requirements because no chemicals addition is required. The acoustic cavitations produce [21] a lot of hot spots of high temperatures and pressures causing H_2O sonolysis. Thus, radical species are formed, which induce the degradation of the organic compound present in the aqueous solution. The polymer scission by the ultrasonic method is unique because the depolymerization proceeds non-randomly at the mid-point of the chain. The radical generation during ultrasonication (US) initiates the depolymerization of polysaccharides especially in case of water-soluble polymers [17, 21].

The combination of US/O_3 has been reported to exhibit several advantages compared to the individual US or O_3 processes. It is selected to enhance the organic compounds oxidation. The degradation of guar gum (GG) is significantly increased by about 99.1 % in case of US/O_3 application [22]. Yue et al. [23] report that the combined US/O_3 treatment results in a significant decrease of chitosan molecular weight. US and O_3 treatment procedures have not been so far applied to the depolymerization of κ-carrageenan. Therefore, this study aims to investigate their individual and combined effects in this case. The physicochemical and morphological properties of the native and the treated κ-carrageenan are analyzed using the barium chloride - gelatin method, FT-IR, SEM and XRD to follow the performance of the processes investigated.

EXPERIMENTAL

Materials

The commercial κ-carrageenan used in this study was provided by CV. Karagen, Indonesia. The carrageenan powder was dissolved in distilled water. A complete dissolution was achieved by heating at 70°C combined with slow stirring. The polysaccharides in the solution were precipitated by a dropwise addition of isopropyl alcohol (E. Merck, Catalog No. 818766) under vigorous stirring. The precipitate of refined κ-carrageenan was collected and dried at 60°C in a forced-air oven. 37 % hydrochloric acid solution (E. Merck, Catalog No. 100314) and sodium hydroxide (E. Merck, Catalog No. 137020) were used to adjust the initial pH value. All the chemicals were used without any pretreatment.

An experimental Set-up

The depolymerization treatment of κ-carrageenan was performed in a transparent glass reactor of a capacity of 500 mL. The refined κ-carrageenan solution (1:100, w/v) was prepared by dissolving it in distilled water at 70°C. The experiments were carried out by different methods: by US only, by O_3 only and by a combination of US/O_3. The sonication process used an ultrasonic generator of 150W and 40 kHz. Different dissolved ozone concentrations (15 mg $min^{-1}L^{-1}$, 25 mg $min^{-1}L^{-1}$, 35 mg $min^{-1}L^{-1}$) were used during the ozonation process. The ozone was produced by an ozone generator (Dipo Technology Indonesia) and bubbled in the solution through a bubble diffuser. The inlet ozone concentration was determined by the indigo colorimetric method. This was done at different times (0 min, 5 min, 10 min, 15 min and 20 min). The initial pH value was adjusted by

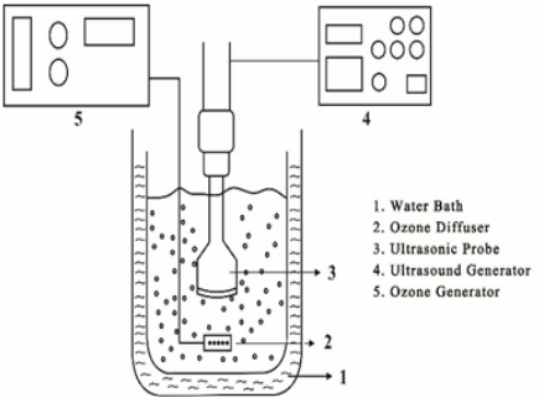


Fig. 2. A schematic presentation of the experimental equipment used for US/O_3 treatment.

the addition of HCl/NaOH, while the temperature of the process was maintained by a water bath. The schematic presentation of the equipment used is shown in Fig. 2.

Analytical Procedures

A Molecular Weight Determination

At regular intervals samples were withdrawn, cooled to 25°C ± 1°C in chilled water to quench the reaction and the intrinsic viscosity was analyzed using a glass capillary Ubbelohde viscometer. The molecular weight of κ-carrageenan was determined according to the Mark-Houwink equation. Lai et al. [13] advanced an equation relating the intrinsic viscosity to the molecular weight as follows:

$$[\eta] = kM_H.M^{\alpha} \quad (1)$$

where [η] was the intrinsic viscosity, kM_H and α were constants, while M was the number-averaged molecular weight. The values of kM_H and α for the κ-carrageenan aqueous solution at 25°C referred to 0.00778 and 0.90, respectively [24, 25]. The intrinsic viscosity was obtained using the Huggins equation:

$$\frac{\eta sp}{C} = [\eta] + kH\,[\eta]^2\,C \quad (2)$$

where ηsp, C, and kH were the specific viscosity, the concentration of the solution, and the Huggins constant of 0.3 [24], correspondingly.

A Sulfate Analysis

The sulfate content of both the non-treated and the treated κ-carrageenan was determined using the barium chloride-gelatin method [26]. A known amount of the sample (W_1, g) was hydrolyzed using 50 mL of 1 N HCl for 30 min by heating at the boiling temperature. Ten milliliters of 0.25 M $BaCl_2$ solution were added to the reaction mixture, which was then cooled to a room temperature and kept for 5 h. The $BaSO_4$ precipitate was filtered with ashless filter paper and incinerated in a muffle furnace at 700°C for 1 h. The ash was weighed (W_2, g) and the sulfate content was calculated in accordance with the equation:

$$\%\ Sulfate = (W_2 / W_1) \times 41.16 \quad (3)$$

FT-IR Spectroscopy

The Fourier transform infrared (FT-IR) spectra of the original and the depolymerized κ-carrageenan were recorded with KBr powder (10 mg κ-carrageenan powder in 90 mg KBr powder) using a Perkin Elmer IR 10.6.1 Spectrophotometer (USA) in the range from 400 cm^{-1} to 2000 cm^{-1}.

X-ray Diffraction (XRD)

The X-ray diffractograms of the original and the treated κ-carrageenan were obtained with an X-ray diffractometer (XRD-7000, Shimadzu, Japan). The scanning region of the diffraction ranged from 10° to 80° with a target voltage of 30 kV, a current of 30 mA and a scan speed of 5°/min. The relative crystallinity (RC) of chitosan was calculated using the equation proposed by Klein et al. [27]:

$$RC\% = \left(\frac{A_c}{A_c + A_a}\right) x100 \quad (4)$$

where A_c was the crystalline area, while A_a was the amorphous area.

Scanning Electron Microscopy (SEM)

The SEM characterization was carried out using a Scanning electron microscope JSM-6510-LA JEOL series (Japan). Prior to the analysis, the original and the treated κ-carrageenan samples were sprinkled onto adhesive Al or C tapes and were supported on metallic discs coated with Au. The images of the sample surfaces were recorded at different magnifications.

RESULTS AND DISCUSSION

A Comparison of Ultrasonication and Ozonation in Respect to κ-Carrageenan Depolymerization

The κ-carrageenan solution is treated by US only and O_3 only at different ozone concentrations. The experiments are carried out at 29±1°C and an initial pH value adjusted at 7. The US and O_3 treatments are applied at 0-th min, 5-th min, 10-th min, 15-th min and 20-th min. The correlation between the molecular weight of κ-carrageenan and the reaction time is illustrated in Fig 3.

The initial molecular weight of κ-carrageenan refers to 550 kDa in all treatment procedures. The decrease of κ-carrageenan molecular weight by US treatment only is marked by the symbol (.). The molecular weight decreases from 550 kDa to 449 kDa within 20 min of ultrasonication. The decrease in this case is found equal to 18.36 %. Aiming to compare the effect of the individual treatment approaches to κ-carrageenan depolymerization, three different concentrations of ozone are used: 15 mg $min^{-1}L^{-1}$ (marked by Δ), 25 mg $min^{-1}L^{-1}$ (marked by ○) and 35 mg $min^{-1}L^{-1}$ (marked by □). The results show that κ-carrageenan molecular weight decreases from 550 kDa to 317 kDa, 288 kDa and 268 kDa within 20 min of ozonation, respectively. Therefore, the decrease observed in this case refers to 42.36 %, 47.64 %, and 51.12 %, correspondingly. Thus, it can be concluded that the percentage decrease of the number-averaged molecular weight of κ-carrageenan in case of an ozone treatment is higher than that obtained by an ultrasonic treatment. Prajapat and Gogate [22] find that the decrease of the intrinsic viscosity of guar gum by an ozone treatment

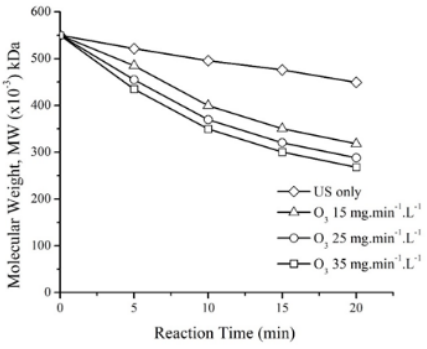


Fig. 3. A dependence of κ-carrageenan molecular weight on the reaction time.

(96.20 %) is only marginally higher when compared to that obtained by an ultrasonic radiation (95.59 %). The ozone treatment is more effective than the ultrasonic radiation in respect to chitosan degradation [23]. It is also reported that the chitosan viscosity is decreased by 12.74 % during an individual ultrasonic radiation. In case of an ozone treatment, it increases within 30 min from 31.18% to 41.38% for an ozone dosage ranging from 35±5 mg/min to 65±5 mg/min. In general, it can be said that the ozone treatment provides a much greater viscosity decrease than that in case of an individual ultrasound treatment.

The reactions proceeding in presence of ultrasonication refer to pyrolysis and chemical interactions with radicals produced from H_2O. The hydroxyl radicals are generated by the sonication of H_2O [23]. The corresponding equation is shown below:

$$H_2O+))) \rightarrow \bullet OH + \bullet H \quad (5)$$

During the ozonation treatment alone, O_3 is transferred from the gas phase into the liquid one. It reacts immediately with the κ-carrageenan solution through direct and indirect interactions with the participation of radicals generated by ozone auto decomposition [18] as shown below:

$$O_3 \rightarrow O_2 + O\bullet \quad (6)$$

$$O\bullet + H_2O \rightarrow 2\bullet OH \quad (7)$$

A Synergetic Effect of US and O_3

The US/O_3 joint application results in a synergetic effect, which leads to an increase of κ-carrageenan depolymerization. Three different ozone concentration (15 mg $min^{-1}L^{-1}$, 25 mg $min^{-1}L^{-1}$, and 35 mg $min^{-1}L^{-1}$) are used. The molecular weight of κ-carrageenan decreases within 20 min of a treatment from 550 kDa to 114 kDa, 84 kDa and 64 kDa, respectively (Fig. 4). The percentage decrease of κ-carrageenan molecular weight in this case refers to 79.27 %, 84.47 %, and 88.36 %, correspondingly. It is intriguing that the average molecular weight decrease observed in case of the joint O_3/US treatment is much higher when compared to that found when US and ozone applications are considered. In addition, the viscosity-averaged molecular weight decrease is much greater under the effect of US/O_3 than that obtained by summation of the degradation values reached by indi-

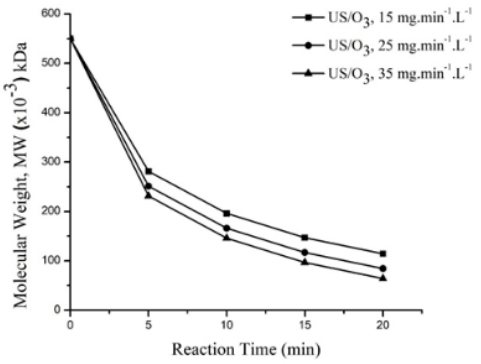


Fig. 4. An effect of the combined ultrasonication and ozonation on κ-carrageenan depolymerization (a temperature of 29±1°C; an initial pH value of 7).

vidual ultrasonic radiation and ozone treatments. The extent of carrageenan degradation obtained by the joint effect of US/O_3 equals 88.36 %, while the value found by summation of those referring to the both processes amounts only to 69.48 % (51.12 % in case of an ozone treatment and 18.36 % obtained by an ultrasonication).

When an organic substance such as carrageenan is treated by ozone in presence of an ultrasonic radiation, hydroxyl radicals are obtained in correspondence with four reaction schemes. The first one refers to H_2O sonication described by Eq. (5). The second one refers to ozone self-decomposition presented by Eqs. (6) and (7). The radiolysis of ozone by the ultrasonic radiation is attributed to the third scheme. It is described by Eqs. (8) and (9):

$$O_3 +))) \rightarrow O_2 + O\bullet \quad (8)$$

$$O\bullet + H_2O \rightarrow 2\bullet OH \quad (9)$$

It provides hydroxyl radicals attacking the β-D-(1,3)-galactose linkages of κ-carrageenan. The fourth reaction scheme refers to oxygen dissociation in the vapor phase of the cavitation bubbles. It results in generation of •OH in correspondence with:

$$O_2 +))) \rightarrow O_2 + O\bullet \quad (10)$$

$$O\bullet + H_2O \rightarrow 2\bullet OH \quad (11)$$

The mechanism of κ-carrageenan depolymerization might be affected by each of the schemes pointed above. This fact agrees with the results of Yue et al. [23] who report that the combined US/O_3 treatment is effective in respect to chitosan degradation. It is so because the hydroxyl radicals obtained are powerful oxidizing species which attack and break the β-D-(1,4)-glucosidic linkages of chitosan.

The ultrasonic radiation can increase the mass transfer of ozone in the κ-carrageenan solution thereby increasing the value of the volumetric mass transfer coefficient. On the other hand, the addition of an ultrasonication radiation during ozonation can produce a turbulent flow leading to the formation of smaller ozone bubbles. The latter have the function of sonolysis cavitation nucleus generating more acoustic cavitations. This facilitates the process of ozone molecules diffusion in κ-carrageenan solution, increasing the contact area and the oxidation rate. The ultrasound treatment enhances the ozone production of •OH, which in turn increases the oxidation rate. Ozone is rapidly decomposed in the vapor phase of the cavitation bubbles during the combined US/O_3 treatment. Furthermore, the ultrasonic radiation also increases the decomposition reaction of ozone, which in turn favors the attack of the β-D-(1,3)-galactose linkages of κ-carrageenan.

An Effect of US/O_3 Operating Conditions

An Effect of pH

Fig. 5 shows the pH effect on carrageenan depolymerization achieved by joint US and O_3 application. The initial pH of the κ-carrageenan solutions studied is adjusted at 3, 7 and 11. Ozone of a concentration of 35 mg $min^{-1}L^{-1}$ is bubbled through them and at the 20-th min the molecular weight of κ-carrageenan is decreased from 550 kDa to 18 kDa, 64 kDa and 87 kDa, respectively. It is worth adding that the percentage of κ-carrageenan depolymerization refers to 96.73 %; 88.36 % and 84.18 %, correspondingly.

The results show that pH has a significant effect on the depolymerization of κ-carrageenan in case of US/O_3 treatment. The reaction becomes faster as pH decreases. It is pointed above that the concentration of H^+ increases under acidic conditions and thus more β-1,4-glycosidic bonds are attacked [28]. Yoon et al. [29] report that the depolymerization of seaweed extracts proceeds like that of cellulose. The glycosidic oxygen in cellulose

β-1,4-glycosidic bonds rapidly interacts with H^+ of the acid to produce a conjugated acid. Therefore, the C-O bond slowly cleavages to form two fragments. One of the fragments has OH linkage as an end group, while the other one ends with a cyclic carbocation.

This research shows that the percentage of κ-carrageenan depolymerization at low pH is higher than that under alkaline conditions. The ozonation reaction is dominated by the direct ozonation process under acidic conditions. On the other hand, an indirect ozonation predominates under neutral and alkaline conditions [6]. Lemeune et al. [19] observe that the decomposition of ozone, which is initiated by $^{\bullet}OH$, produces hydroxyl radicals that can very rapidly attack glucose or cellobiose with low reaction selectivity. Chirat and Lachenal [30] conclude that the molecular ozone is mainly responsible for the cellulose degradation and that the cellulose is less degradable at high pH.

The effect of pH on the average molecular weight can also be outlined in terms of the reaction rate as well as the amount of the hydrolysis product. Lenihan et al. [31] hydrolyze a lignocellulosic biomass from potato peel using phosphoric acid of various concentrations, i.e. 2.5 wt.%, 5.0 wt.%, 7.5 wt.%, and 10 wt.%. The latter are equivalent to pH of 1.40, 1.23, 1.14, and 1.10, respectively. They report that the reaction rate increases with the acid concentration increase or pH decrease. Kumar et al. [32] who hydrolyze sugar cane bagasse using sulfuric acid as a catalyst report that the hydrolysates (xylose, glucose, and furfural) increase as the percentage of the sulfuric acid increases. Myslabodski et al. [33] who hydrolyze κ-carrageenan solutions of various pHs at different temperatures report similar results. They find that the 25 % decrease of the molecular weight in case of hydrolysis at pH of 6, 5, 4, and 3 requires 12 days, 2 days, 8 h, and 1.4 h, respectively.

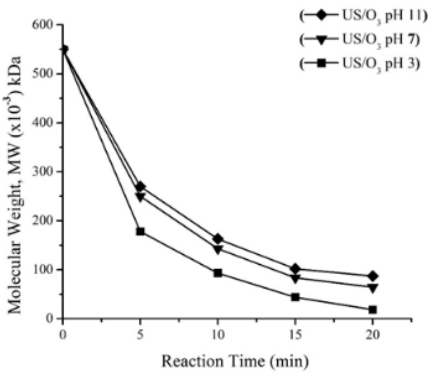


Fig. 5. An effect of pH on κ-carrageenan depolymerization (a temperature of 29±1°C, O_3 concentration of 35 mg $min^{-1}L^{-1}$).

A Temperature Effect

The effect of US/O_3 treatment on κ-carrageenan depolymerization taking place within the temperature range of 30°C -50°C is illustrated in Fig. 6.

The reaction starts at an initial pH value of 3 and an ozone concentration of 35 mg $min^{-1}L^{-1}$. The temperature is adjusted at 30◦C, 40◦C and 50 ◦C. The viscosity-averaged molecular weight of κ-carrageenan decreases from 550 kDa to 18 kDa, 16 kDa and 12 kDa, correspondingly. The depolymerization of κ-carrageenan increases slightly from 96.73 % to 97.82 % in case of 20 min of US/O_3 treatment and a reaction temperature increase from 30°C to 50°C.

The combined US/O_3 treatment has a positive and negative effect on κ-carrageenan depolymerization. The ozone concentration in the κ-carrageenan solution deceases with the reaction temperature increase. On the other hand, the later leads to an increase of the mass transfer rate during the ozonation process. Simoes and Castro [34] find that the depolymerization rate of hollocellulose increases markedly with the temperature increase.

An Effect of US/O_3 Treatment on κ-Carrageenan Sulfate Content

The low molecular weight and sulfated derivatives of κ-carrageenan exhibit beneficial biological activities. The sulfate content remaining in the treated κ-carrageenan is calculated using the barium chloride - gelatin method. As shown in Fig.7, the sulfate content after US, O_3 and US/O_3 treatment does not significantly decrease. The sulfate content of the native carrageenan is equal to 13.87±0.25 %. After 20 min of US/O_3 treatment at pH values of 11, 7 and 3 the sulfate content is decreased to 12.03±0.25 %, 11.78±0.19 %, and 11.50±0.27 %, respectively. The results of this study suggest that the sulfate groups content is slightly decreased during the US/O_3 process taking place at different pH values. Other researchers [8, 14, 15, 35] find that κ-carrageenan is relatively stable during the depolymerization process.

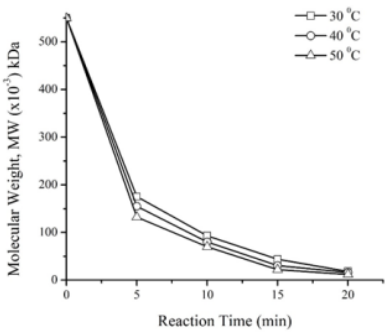


Fig. 6. A temperature effect on κ-carrageenan depolymerization (an initial pH value of 7 and O_3 concentration of 35 mg min^{-1}L^{-1}).

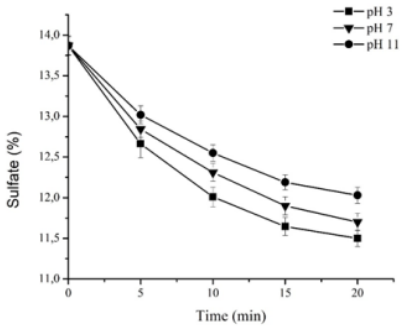


Fig. 7. A sulfate content of κ-carrageenan upon US/O_3 treatment at various pH values.

The sulfate residues obtained after an irradiation amount approximately to 90 %, 83 % and 71 % in case of κ-, ι-, and λ- carragenan, respectively [15].

Sun et al. [36] carry out a comparative investigation of the sulfate content of κ-carrageenan remaining after H_2O_2 depolymerization, enzymatic digestion, partial reductive hydrolysis, and HCl hydrolysis. The corresponding values obtained refer to 15.47 ± 0.43 %, 15.27 ± 0.35 %, 15.55 ± 0.16 %, and 11.87 ± 0.51 % in case of an initial sulfate content of 15.66 ± 0.30 %. Karlsson et al. [14] observe no significant change of the sulfate content when κ-carrageenan depolymerization is achieved by mild acidic hydrolysis. Prasetyaningrum et al. [37] also observe that the sulfate content of κ-carrageenan does not significantly decrease during the ozonation.

The biological activity of the low molecular weight products of κ-carrageenan depolymerization depends on their chemical structure, molecular weight, and chain conformations. Wang et al. [6] find that the oligosaccharides of κ-carrageenan and their sulfated derivatives effectively inhibit the influenza A virus. The degree of sulfation and the molecular weight are the main factors affecting the activity of κ-carrageenan oligosaccharides in this respect. The most active κ-carrageenan oligosaccharide has a sulfate content of 0.8 - 1.0 mole/mole of a disaccharide and a molecular weight of 1 kDa - 3 kDa [6]. Yuan and Song [9] study the depolymerization of κ-carrageenan by acid hydrolysis and test the activity of its products in the biomedical field. The bioassay tests show that κ-carrageenan oligosaccharides of a molecular weight of 1.2 kDa and 8.98 % sulfate content have a relatively high antitumor activity compared to that of carrageenan polysaccharide. The extract of low molecular weight fragments of κ-carrageenan of an average molecular weight (Mw), ranging from 2.3 kDa to 5.0 kDa and obtained by a gamma irradiation, exhibit antioxidant properties [38, 39]. Relleve et al. [35] report that the oligomer of a molecular weight of 10 kDa obtained from κ-carrageenan shows a strong growth promotion effect on potatoes in a tissue culture. The oligoalginate of a molecular weight of approximately 14.3 kDa prepared by an irradiation is found to have the highest positive effect on the propagation of flower plants in a tissue culture [40]. The oligo κ-carrageenan of a molecular weight of approximately 12 kDa - 18 kDa and a sulfate content ranging from 11.50 % to 12.03 % (found in the present investigation) shows an effect in respect to vitro propagation in a tissue culture.

FT-IR Analyses

The infrared spectroscopy method is utilized to follow the structure modification of κ-carrageenan resulting from the chemical depolymerization. The FT-IR spectra of the native κ-carrageenan and that degraded by the methods studied are shown in Fig. 8. The characteristic absorption peaks of κ-carrageenan appearing in the range from 1220 cm^{-1} to 1440 cm^{-1} are determined by the presence of ester sulfate (S=O). The peaks at wavelengths ranging from 1010 cm^{-1} to 1080 cm^{-1}, 928 cm^{-1} to 930 cm^{-1}, 840 cm^{-1} to 850 cm^{-1} are attributed to the presence of glycosidic linkage, 3,6-AG galactose-4-sulfate, and galactose-4 sulfate, respectively. The results of the FT-

IR spectra show that the molecules of the native and the treated κ-carrageenan are quite similar. The peak absorption at 928 cm^{-1} is characteristic of C-O-C in 3,6 anhydro-D-galactose-4-sulfate. The absorption peak of the treated κ-carrageenan at 840 cm^{-1} shows the presence of sulfate groups. The sulfate content of κ-carrageenan is more stable than that of i-carrageenan and λ-carrageenan obtained by an acid hydrolysis process [14]. Relleve et al. [41] observe the depolymerization of κ-carrageenan proceeding in case of a gamma X-ray radiation. It is reported that the sulfate groups are not preferentially or selectively removed. About 90 %, 83 % and 71 % of the sulfate groups remain in the irradiated κ-, i- and λ-carrageenan, respectively.

The wavelength range from 1010 cm^{-1} to 1080 cm^{-1} is assigned to the glycosidic bond of κ-carrageenan. The results of this study show that there is a decrease of the intensity at 1027 cm^{-1} for the treated κ-carrageenan. This indicates a cleavage of β-1,4-glycosidic linkages of κ-carrageenan after US/O_3 treatment. Relleve et al. [42] observe that important functional groups are retained after an irradiation. There is a considerable decrease of the peak heights corresponding to the glycosidic, 3,6 anhydrogalactose and methylene groups of κ-carrageenan after the radiation treatment. Li et al. [43] also report that there is a decrease of the intensity of the FT-IR peaks referring to degraded polysaccharide rhamnogalacturonan obtained by a combined hydrogen peroxide oxidation and an ultrasound treatment. The FT-IR results of this investigation clearly show that there is no significant change of the functional groups and the chemical structure of κ-carrageenan during the US/O_3 treatment. The free radical-induced chain scission results in a molecular weight decrease without any changes of κ-carrageenan activity or functional properties.

X-ray Diffraction Analyses

The X-ray diffraction pattern of the native κ-carrageenan and that treated by US/O_3 are presented in Fig. 9. The diffraction patterns illustrated show a similar pattern with main peaks at 43.88°, 64.23° and 77.46°. Elsupikhe et al. [44] find similar results. This research shows that there is a decrease of the main peak of the treated κ-carrageenan when compared to that of the native one. Prasetsung et al. [45] report that the chitosan degraded by a plasma treatment shows a lower intensity of the main peak than the native one. The relative crystallinity (RC) of the treated κ-carrageenan (57,03 %) is lower than that of the native κ-carrageenan (72,54%). These results indicate that the crystal structure of the treated κ-carrageenan is destroyed during the US/O_3 treatment. Moreover, the treated κ-carrageenan has an amorphous structure. Rokhati et al. [46] also observe a decrease of RC after chitosan is subjected to a degradation.

SEM Analysis

The surface topography of solid samples of a native κ-carrageenan and that subjected to US/O_3 treatment is obtained using SEM (Fig.10). The results indicate that the surface morphology of the treated κ-carrageenan is relatively rougher and more amorphous than that of the native κ-carrageenan. This finding agrees with the data

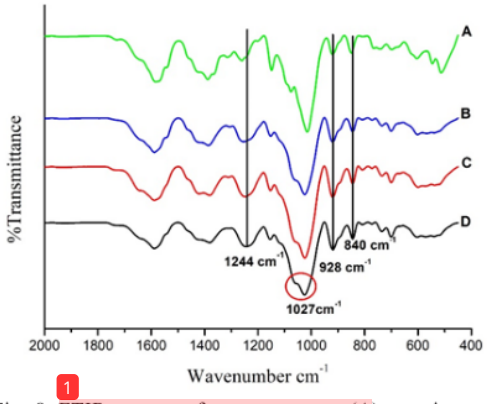


Fig. 8. FTIR spectra of κ-carrageenan: (A) a native one; κ-carrageenan treated by US (B), by O_3 (C) and by US/O_3 (D).

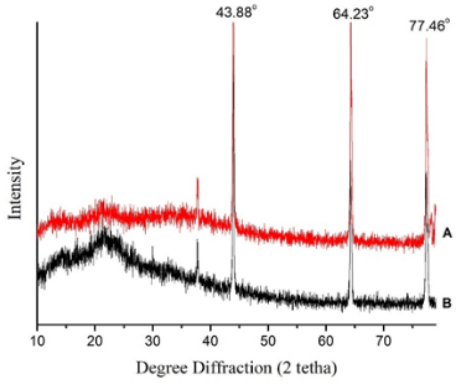


Fig. 9. X-ray diffraction patterns: (A) native κ-carrageenan, (B) treated κ-carrageenan.

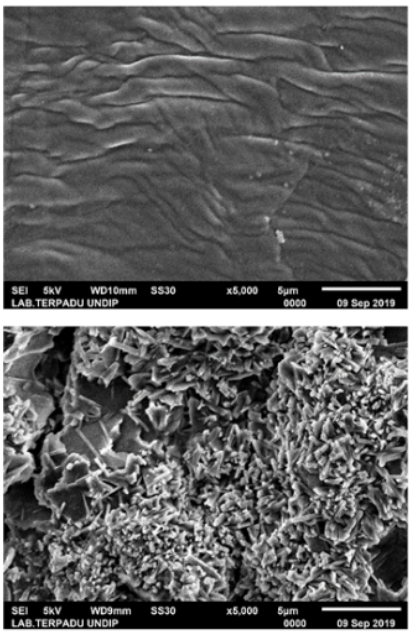


Fig. 10. SEM results referring to: (A) native κ-carrageenan; (B) κ-carrageenan treated by US/O_3.

of Fojas et al. [47] who report that the surface morphology of κ-carrageenan subjected to an irradiation treatment is amorphous and rough. Shahbazi et al. [48] also observe that the thermal degradation of κ-carrageenan results in a rougher and more porous surface, while the structure becomes amorphous and of an irregular shape. This is due to the cleavage of the glycosidic linkage of κ-carrageenan during the depolymerization process.

CONCLUSIONS

κ-carrageenan is efficiently depolymerized by ozone in presence of an ultrasonic radiation. The combined treatment applied provides a synergetic effect. The medium pH value has a significant effect on the depolymerization process unlike that of the temperature increase. There is also no significant change of the sulfate groups content in case of depolymerization by US, O_3, and US/O_3. The FT-IR analysis of the native and the degraded κ-carrageenan shows that there is no significant change of the functional properties. The XRD analysis indicates a decrease of the main peak of the treated κ-carrageenan when compared to that of the native one. The κ-carrageenan studied becomes amorphous and rough as a result of the treatment applied.

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