Degradation and Antioxidant Activity of κ-Carrageenans

Tao Sun, Huina Tao, Jing Xie, Shuo Zhang, Xin Xu

College of Food Science, Shanghai Ocean University, Shanghai 201306, China

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ABSTRACT: κ-Carrageenan was degraded by an oxidative method involving hydrogen peroxide (H_2O_2). The molecular weight was measured by gel permeation chromatography. The effects of the concentration of H_2O_2 and initial κ-carrageenan, pH value, and degradation time on the molecular weights of the degraded products were studied. The structural change of the degraded κ-carrageenans was characterized by Fourier transform infrared spectroscopy and determination of the sulfate content. The antioxidant activity of the degraded κ-carrageenans was evaluated as scavengers of superoxide anions and hydroxyl radicals by application of flow injection chemiluminescence technology. The values of the 50% inhibition concentration (IC_{50}) against the superoxide anion of degraded κ-carrageenans labeled A, B, C, and D (with weight-average molecular weights of 3250, 5820, 15,080, and 209,000, respectively) were 2.65, 3.22, 6.66, and 8.13 mg/mL, respectively. As for hydroxyl radical scavenging, the IC_{50} values of κ-carrageenans A, B, C, and D were 0.014, 0.049, 0.062, and 0.110 mg/mL, respectively. The results indicated that the κ-carrageenans with lower molecular weights had better antioxidant activity. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 194–199, 2010

Key words: antioxidants; degradation; polysaccharides

INTRODUCTION

Carrageenans are sulfated anionic polymers composed of D-galactose units linked alternately with α-1,4 and β-1,3 linkages.[1] They are classified according to the presence of 3,6-anhydrogalactose on the 4-linked residue and the number and position of the sulfated groups.[2] κ-Carrageenan is one kind of carrageenan and is widely used in the food industry because of its economy and importance. It has been found to have valuable biological functions.[3–6] However, further utilization has been limited because of its high molecular weight and viscosity properties. Carrageenan oligosaccharides can be prepared by chemical, physical, and enzymatic degradation.[7–11] Recently, carrageenan oligosaccharides and their different derivatives have been reported to play an important role as free-radical scavengers *in vitro* and antioxidants for the prevention of oxidative damage in living organisms and to show antitumor activity.[12–14]

However, the relationship between the molecular weights of κ-carrageenan oligosaccharides and antioxidative activity has received less attention. To investigate the effect of the molecular weight on the antioxidant activity, κ-carrageenans of different molecular weights were prepared by oxidative degradation, and the antioxidant activity of degraded κ-carrageenans were evaluated against superoxide anions and hydroxyl radicals.

EXPERIMENTAL

Chemicals

κ-Carrageenan [weight-average molecular weight (M_w) = 3.5×10^5] was purchased from Shanghai United Food Additives Co., Ltd. (Shanghai, China). Luminol was a Sigma reagent (Shanghai, China). All other chemicals were analytical grade and were supplied by Shanghai Chemicals Co. (Shanghai, China)

Degradation of the κ-carrageenans

κ-Carrageenan was oxidatively degraded with hydrogen peroxide (H_2O_2) according to a literature procedure with some modifications.[15] κ-Carrageenan (2.0 g) was added to 80.0 mL of distilled water, then heated to 80°C, and stirred until a homogeneous solution was formed. The pH value of the κ-carrageenan solution was adjusted from 1.0 to 13.0 with a 2.0 mol/L HCl solution or 2.0 mol/L NaOH solution. After preequilibrium in an 80°C water bath, 20.0 mL of H_2O_2 solution (30%, w/w) was dropped into the κ-carrageenan solution within 30 min. After degradation for 4 h, the κ-carrageenan solution was added to

Correspondence to: T. Sun (taosun@shou.edu.cn).
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three-amount ethanol to cause a precipitation. The precipitation was centrifuged at 3000 rpm for 10 min, collected, and then refined three times by the dissolution–precipitation process. Finally, the precipitation was collected and dried *in vacuo* to obtain degraded κ-carrageenan. The amount of initial κ-carrageenan and the volume of the H_2O_2 solution were changed to obtain degraded products of different molecular weights. The structural change of the degraded κ-carrageenans was confirmed in a Fourier transform infrared (FTIR) spectrometer (Nicolet NEXUS 470, USA). The sulfate content was determined according to method similar to that of Dodgson and Price.[16] κ-Carrageenans were hydrolyzed with HCl to prepare a hydrolysate. The hydrolysate (0.5 mL, 0.2 mg/mL) was mixed homogeneously with trichloroacetic acid (0.35 mL, 8%) and barium chloride/gelatin reagent (0.25 mL, 5 mg/mL) and was then kept at room temperature for 15–20 min. The absorbance of this test solution at 360 nm (A_1) was then measured against a blank solution with deionized water substituting κ-carrageenan hydrolysate. The control solution was prepared according to the previous procedure with a gelatin solution (0.35 mL, 5 mg/mL) substituting the barium chloride/gelatin reagent, and the absorbance at 360 nm (A_2) was measured. The calibration curve was prepared by the absorbance of a series of standard solutions (potassium sulfate of different concentrations substituting κ-carrageenan hydrolysate). The sulfate content in the κ-carrageenans was obtained from the calibration curve corresponding to the point of $A_1 - A_2$.

Molecular weight determination of the degraded κ-carrageenans

Molecular weights of the initial and degraded κ-carrageenans were measured by a similar gel permeation chromatography method with glucan as a standard.[17] It was used to estimate the average molecular weights of the κ-carrageenans. Gel permeation chromatography determination of the degraded κ-carrageenans was performed on a Waters 515 chromatograph equipped with a Waters (USA) 2410 refractive-index detector and Ultrahydrogel 500 and 120 columns. The analysis was carried out with a 0.07% Na_2SO_4 solution as the mobile phase at a flow rate of 0.5 mL/min. The temperatures of the column and detector were both maintained at 40°C during the determination process.

Antioxidant activity assays

Antioxidant activity of the degraded κ-carrageenans was evaluated by the scavenging of superoxide anions and hydroxyl radicals on a biochemical luminometer (IFFDM-D, Xi'an, China).[18] Superoxide anion was produced by a luminol-enhanced autoxidation of pyprogallol. The chemiluminescent reaction was processed in a Na_2CO_3–$NaHCO_3$ (pH = 10.20, 0.05 mol/L) buffered solution at room temperature. The final concentration of luminol was 4×10^{-4} mol/L, and that of pyprogallol was 5×10^{-5} mol/L. Degraded κ-carrageenans labeled A, B, C, and D (with M_w values of 3250, 5820, 15,080, and 209,000, respectively) were dissolved in an Na_2CO_3–$NaHCO_3$ buffered solution to prepare scavenger solutions at different concentrations from 3.6×10^{-3} to 3.0 mg/mL. The scavenging activity of the κ-carrageenans against superoxide anions was evaluated according to their quenching effects on the chemiluminescence signal of the luminol–pyrogallol system. The inhibiting efficacy for the superoxide anion was calculated as follows:

$$\text{Inhibiting efficacy}\,(\%) = (A_0 - A_i)/A_0$$

where A_0 and A_i represent the chemiluminescence peak areas of the blank group and test group, respectively. The free-radical produced in the system was proven to be superoxide anion tested by superoxide dismutase, catalose, and mannitol. Ascorbic acid was used as a control.

Hydroxyl radical scavenging activity was processed by a program similar to that described previously. Hydroxyl radical was produced in a Fe(II)–H_2O_2–luminol system. The chemiluminescent reaction was processed in K_2HPO_4–NaOH (pH = 7.4, 0.05 mol/L) buffered solution at room temperature. The final concentrations were $[K_4Fe(SCN)_6] = 0.8$ mg/mL, $[H_2O_2] = 0.012$ mol/L, and [Luminol] = 6.4×10^{-4} mol/L. The hydroxyl radical was proven by superoxide dismutase, catalase, and mannitol. Ascorbic acid was used as a control.

RESULTS AND DISCUSSION

Changes in the chemical structure

The structural change of initial κ-carrageenan and the degraded κ-carrageenans D and A ($M_w = 3.5 \times 10^5$, 209,000, and 3250, respectively) were confirmed by FTIR technology. As shown in Figure 1, the characteristic absorption peaks appearing at 1260 and 850 cm^{-1} were attributed to S=O of the sulfate esters and C—O—S of the axial secondary sulfate on C-4 of galactose, respectively. The band at 930 cm^{-1} was a characteristic absorption of C—O of 3,6-anhydro-D-galactose. These characteristic peaks of degraded κ-carrageenans D and A indicated that the main structure of the κ-carrageenan was not destroyed in the degradation process. However, compared to the spectra of initial κ-carrageenan and

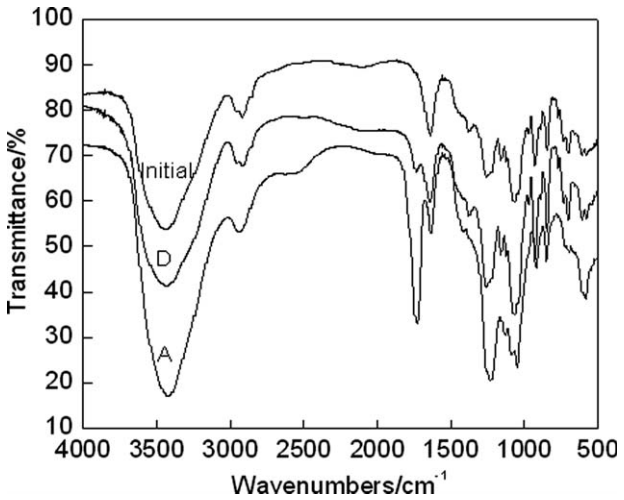

Figure 1 FTIR spectra of the initial κ-carrageenan ($M_w = 3.5 \times 10^5$) and degraded κ-carrageenans D ($M_w = 209, 000$) and A ($M_w = 3250$).

D, the absorption spectrum of κ-carrageenan A was relatively simple, especially the absorption at 1000–700 cm^{-1}. Some small peaks, such as 970 cm^{-1} (glycoside bonds) and 890 cm^{-1} (C_6 group in β-D-galactose), disappeared in the spectrum of κ-carrageenan A.[19] The absorption peaks at 1150–1100 cm^{-1} became relative stronger and moved to a low wave number, which was probably due to the partial destruction of the hydrogen bonds in the polysaccharide chain. A new absorption band appeared at 1736 cm^{-1} (D) or 1732 cm^{-1} (A), and its intensity increased with decreasing molecular weight of the degraded κ-carrageenans. These facts suggested the formation of a carbonyl group in the degraded κ-carrageenans.[17,20,21]

Compared with initial κ-carrageenan, the degraded products had the following different characteristics:

1. The molecular weights decreased, and thus, they had short polysaccharide chain, and the ability to form intramolecular hydroxyl bonds declined sharply, which was confirmed by the fact that the absorption peaks at 1150–1100 cm^{-1} became relative stronger and moved to a lower wave number.
2. A new carbonyl group was formed in degraded κ-carrageenans because of the oxidation of C—O—C, which was confirmed by the new absorption peak at about 1736 cm^{-1} in FTIR spectra.
3. The sulfate content in the polysaccharide decreased.[20,21] With decreasing molecular weight, the sulfate content of the initial and degraded κ-carrageenans decreased as follows: initial, 20%; D, 17.8%; C, 17.3%; B, 15.4%; and A, 13.7%.

Degradation of the κ-carrageenans

The degradation of polysaccharides can easily be carried out either by chemical or enzymatic hydrolysis. Like other polysaccharides, κ-carrageenan is susceptible to a variety of degradation techniques, including acid hydrolysis, radiation degradation, and enzymatic hydrolysis.[22] Recently, oxidative degradation has been studied.[23,24] Oxidative degradation involving H_2O_2 has been widely applied in the degradation of other polysaccharides.[25,26] The technique is based on the formation of a reactive hydroxyl radical by the disassociation of H_2O_2. Hydroxyl radicals, which are powerful oxidizing species, can attack the glucosidic linkages of κ-carrageenan.

In this study, κ-carrageenan was degraded by using H_2O_2. We prepared a serial of degraded products with different molecular weights by changing the reaction conditions. The effects of the following factors on degradation were investigated: the concentration of H_2O_2, concentration of κ-carrageenans, and pH value of the reaction system. Four degraded κ-carrageenans, A, B, C, and D ($M_w = 3250$, 5820, 15,080, and 209,000), were selected for evaluation of antioxidant activity. They were prepared by variation of the reaction conditions, as shown in Table I.

Effect of the concentration of H_2O_2

κ-Carrageenan was oxidatively degraded, and the effects of the concentration of H_2O_2 on degradation were evaluated with fixed conditions: [κ-Carrageenan] = 25 mg/mL, pH 7.0, and degradation time = 4 h. When the concentration of H_2O_2 was changed

TABLE I
Reaction Conditions for the Preparation of Degraded κ-Carrageenans A, B, C, and D

Degraded κ-carrageenan	Concentration of the initial κ-carrageenan (mg/mL)	Concentration of H_2O_2 (mg/mL)	pH value	Degradation time (h)
A ($M_w = 3250$)	25	3.75	7.0	4
B ($M_w = 5820$)	25	1.50	7.0	4
C ($M_w = 15,080$)	25	1.50	11.0	4
D ($M_w = 20,900$)	12	0.15	7.0	4

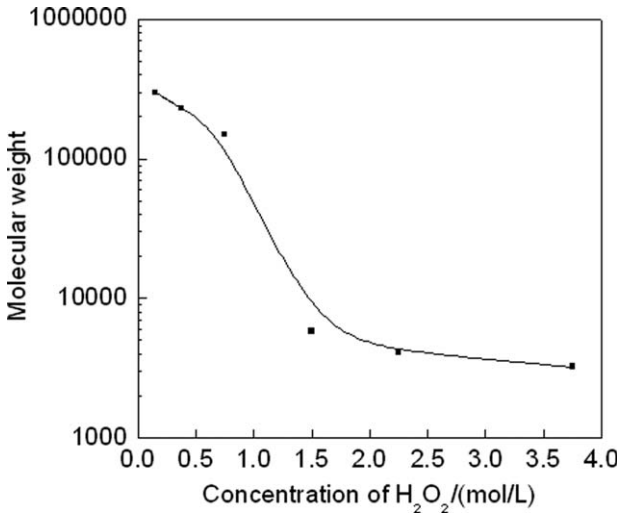

Figure 2 Effect of the concentration of H_2O_2 on the degradation of κ-carrageenan.

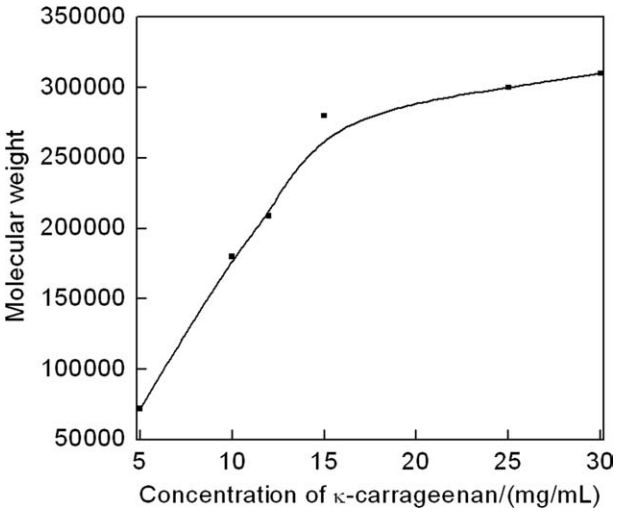

Figure 3 Effect of the concentration of the initial κ-carrageenan on the degradation of κ-carrageenan.

under the previous fixed conditions, the higher the concentration of H_2O_2 was, the smaller the degraded product molecular weight was. The molecular weights of the degraded κ-carrageenans decreased from 3.0×10^5 to 1.5×10^5 when the concentration of H_2O_2 changed from 0.15 to 0.75 mol/L (Fig. 2). With continuous increasing H_2O_2 concentration, the effect on degradation was not so obvious, and the molecular weights of the κ-carrageenans changed from 5820 to 3250 when the H_2O_2 concentration changed from 1.5 to 3.75 mol/L. H_2O_2 is a unstable oxidant and can disassociate to hydroxyl radical, which is a powerful oxidizing species that can attack the glucosidic linkages of κ-carrageenan. More hydroxyl radicals could be formed at high concentrations of H_2O_2, and the collision chance between hydroxyl radicals and the polymer chain of κ-carrageenan increased, and this resulted in a decrease in the molecular weights of the degraded products.

Effect of the κ-carrageenan concentration

The effect of the concentration of initial κ-carrageenans on the degradation of the κ-carrageenans is shown in Figure 3. The molecular weights of the degraded κ-carrageenans increased with increasing of concentration of initial κ-carrageenans under a fixed H_2O_2 concentration of 0.15 mol/L, a constant degradation time of 4 h, and a pH of 7.0. When the concentration of κ-carrageenans changed from 5 to 12 mg/mL, the effect of κ-carrageenan concentration on degradation was significant, and the molecular weights of the degraded products changed from 7.2×10^4 to 2.1×10^5. The molecular weights of the degraded products were 3.0×10^5 and 3.1×10^5 when the κ-carrageenan concentrations were 25 and 30 mg/mL, respectively. The results indicate that the degradation were processed more easily at a lower concentration of initial κ-carrageenans, and low-molecular-weight κ-carrageenans were obtained.

Effect of the degradation time

Figure 4 shows the effect of the degradation time on the degradation of the κ-carrageenans under fixed conditions: [κ-Carrageenan] = 25 mg/mL, [H_2O_2 concentration] = 0.75 mol/L, and pH = 7.0. The molecular weights of the degraded κ-carrageenans changed from 3.4×10^5 at 1 h to 3.3×10^5 at 2 h. With increasing degradation time, the κ-carrageenan chain was attacked more times, and the molecular weights changed greatly. Finally, a degraded κ-

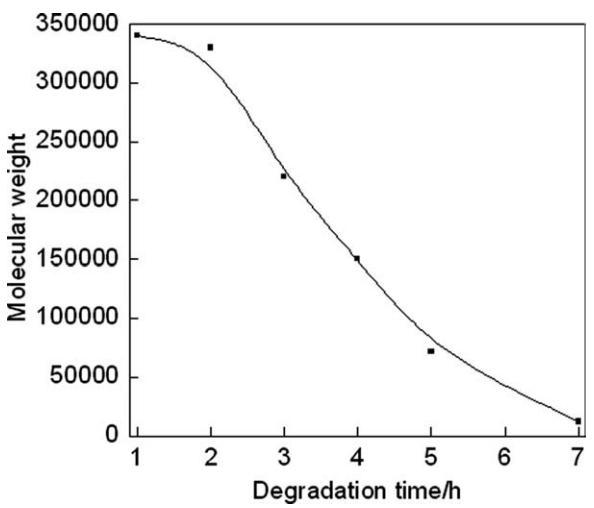

Figure 4 Effect of the degradation time on the degradation of κ-carrageenan.

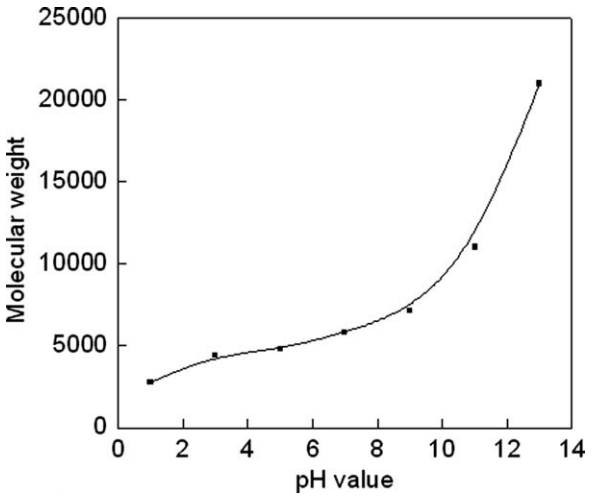

Figure 5 Effect of the pH value on the degradation of κ-carrageenan.

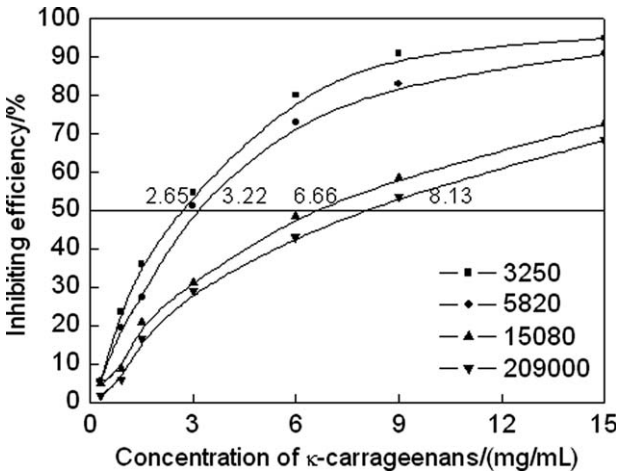

Figure 6 Scavenging effect of degraded κ-carrageenans on the superoxide anion.

carrageenan with an M_w of 1.2×10^4 was obtained when the degradation time was 7 h. The facts indicated that a long degradation time was necessary to obtain low-molecular-weight κ-carrageenans.

Effect of the pH value

The effect of the pH value on the degradation of κ-carrageenans was significant. As shown in Figure 5, the molecular weights of the κ-carrageenans were 2.1×10^5 degraded at pH = 13 and then decreased to 1.1×10^4 at pH = 11 and 2800 at pH = 1. The molecular weights of the degraded κ-carrageenans decreased with decreasing pH value. Under acidic conditions, the oxidative ability of the H_2O_2 was strong, which was useful for the complete degradation of the κ-carrageenans. On the other hand, acidic degradation and oxidative degradation occurred at the same time when the pH value was low.[27]

Antioxidant activity assays

Superoxide anion is a highly toxic species that is generated by numerous biological and photochemical reactions.[28] It may decompose to form stronger, reactive oxidative species, such as singlet oxygen and hydroxyl radicals.[29] Among the reactive oxygen species, the hydroxyl radical is the most reactive and dangerous and can easily react with biomolecules, such as amino acids, proteins, and DNA.[30] The scavenging effects of the degraded κ-carrageenans on superoxide anions and hydroxyl radicals was evaluated by chemiluminescence technology. Figure 6 shows the superoxide anion inhibiting efficacy of the κ-carrageenans A, B, C, and D (M_w = 3250, 5820, 15,080, and 209,000) at different concentrations. All of the samples exhibited various degrees of antioxidant activity. The 50% inhibition concentration (IC_{50}) of 2.65 mg/mL is shown in the figure because a superoxide anion inhibiting efficacy of approximately 50% was achieved at this concentration. As for κ-carrageenans B, C, and D, values of IC_{50} against the superoxide anion were 3.22, 6.66, and 8.13 mg/mL, respectively. At the final concentration of 15 mg/mL in the test system, the maximal inhibiting efficacy of superoxide anion by A, B, C, and D were 95, 90.9, 72.7, and 68.4%, respectively. The results show that the low-molecular-weight κ-carrageenans had a relatively stronger scavenging ability against superoxide anion.

The concentration dependence relations of the inhibiting efficacy of hydroxyl radicals by κ-carrageenans A, B, C, and D are shown in Figure 7. The inhibiting efficacy increased sharply then slightly

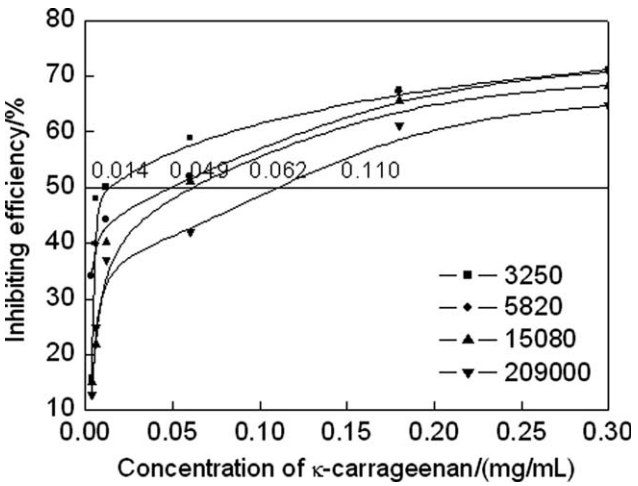

Figure 7 Scavenging effect of degraded κ-carrageenans on the hydroxyl radical.

with increasing concentration. The IC_{50} values of κ-carrageenans A, B, C, and D were 0.014, 0.049, 0.062, and 0.110 mg/mL, respectively. At the final concentration of 0.30 mg/mL in the test system, the maximal inhibiting efficacy of hydroxyl radicals by κ-carrageenans A, B, C, and D were 71.1, 70.8, 68.3, and 64.8%, respectively. Similar to superoxide anion scavenging, the results also indicate that low-molecular-weight κ-carrageenans had a relatively stronger scavenging effect on hydroxyl radicals.

Studies on the antioxidant activity of other polysaccharides and their degraded products have shown that the active hydroxyl and amino groups in the polymer chains may take part in free-radical scavenging and contribute to the antioxidant activity.[31,32] As for κ-carrageenans, the antioxidant activity may also be owed to the hydroxyl groups in the polymer chain. The initial κ-carrageenan showed no scavenging activity against superoxide and hydroxyl radicals in these antioxidant evaluation systems. κ-Carrageenans with lower molecular weights had relatively stronger antioxidant activities. Compared with the initial κ-carrageenan, the degraded products had new carboxyl groups and relatively low contents of sulfate groups. At low molecular weights, the degraded κ-carrageenans had short polymer chains, and their ability to form intramolecular hydrogen bonds declined sharply. That is, the hydroxyl groups were activated, and this was helpful to the radical scavenging process. Second, the sulfate groups decreased and were substituted by hydroxyl groups during the degradation, which meant that the degraded products had more hydroxyl groups. Finally, the new carboxyl groups in the degraded κ-carrageenan were withdrawing groups. This reduced the electron cloud density in the κ-carrageenan chain and enhanced the activity of hydroxyl groups, which thus made it easier to react with superoxide anions and hydroxyl radicals. From these results, we concluded that the low-molecular-weight κ-carrageenans had strong antioxidant activity.

In the antioxidant activity evaluation system, ascorbic acid was used as a control, and its IC_{50} of ascorbic acid was 0.15 mg/mL for superoxide anions and 0.010 mg/mL for hydroxyl radicals. Compared with ascorbic acid, κ-carrageenans with low molecular weights had similar hydroxyl radical scavenging ability and worse superoxide anion scavenging ability. With regard to their natural occurrence and biocompatibility, the antioxidant activity of degraded κ-carrageenans will be helpful for expanding their applications in biomedicine.

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