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Degradation of κ-carrageenan by hydrolysis with commercial α-amylase

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ABSTRACT

κ-Carrageenan was degraded by hydrolysis using commercial α-amylase (4000 U/mg). The hydrolysis process was monitored by the intrinsic viscosity [η] of the hydrolysates. Factors affecting the enzymatic hydrolysis of carrageenan were investigated, and the optimum hydrolysis conditions were as follows: duration, 4 h; pH, 7.5; temperature, 50 °C; and amount of commercial α-amylase, 40 mg of the mixture containing 5 g κ-Carrageenan. Under the optimized conditions, minimum intrinsic viscosity (12.31) was obtained. The dextrose-equivalent value of the resulting products was 20.41, indicating that the average degree of polymerization was approximately equal to 5.0. The hydrolysates were filtered, concentrated to ~15% (w/v), and precipitated with 6 volumes of ethanol; the precipitates were then freeze-dried to yield a white, water-soluble powder. The carrageenan-derived oligosaccharide content of the product and the yield were 96.5% and 92.6% (w/w), respectively.

1. Introduction

Carrageenans are sulfated galactans that comprise the main structural polysaccharides in red seaweed (Rhodophyceae) (Guibet, Kervarec, Génicot, Chevolot, & Helbert, 2006; Relleve et al., 2005). They have been used widely as an ingredient in the food industry to improve the texture of food products, due to their effect of thickening, stabilizing, or emulsifying dairy products, salad dressings, infant formulas, processed meat, soymilk, and other food products (Chen, Yan, Wang, Xu, & Zhang, 2010).

Carrageenans consist of D-galactose residues linked by alternating α-1,3 and β-1,4 glycosidic bonds. Depending on both the presence of a 3,6-anhydro bridge in the α-L,4-linked galactose residue and the position of sulfate substituents, they are referred to as κ-, ⌊-, or λ-carrageenans. κ-Carrageenan is an alternating galactan of 1,3-linked β-D-galactopyranose 4-O-sulfate and 1,4-linked 3,6anhydro-α-D-galactopyranose, and it occurs in the cell wall of certain species of red algae, such as *Chondrus* sp., *Gigartina* sp., *Eucheuma* sp., and *Iridaea* sp. (Liu, Li, Chi, & Chi, 2011).

In addition, carrageenans have many biological functions; however, their further utilization in nonfood applications has been limited by their superior gelling and viscosity properties. Carrageenan-derived oligosaccharides have been shown to elicit the activation of marker enzymes of the defence metabolism in the cells and protoplast of Rubus. They have also been reported to have activities against the human immunodeficiency virus (Relleve et al., 2005) and a few tumors (Mou, Jiang, & Guan, 2003). Carrageenan-derived oligosaccharides can be produced by acid hydrolysis (Myslabodski, Stancioff, & Heckert, 1996; Singh & Jacobsson, 1994), oxidative degradation (Chen et al., 2010), radiation-induced degradation (Relleve et al., 2005), microwave-induced degradation (Zhou, Yao, & Wang, 2006), and enzymatic hydrolysis using carrageenase (Guibet et al., 2006; Jouanneau, Boulenguer, Mazoyer, & Helbert, 2010; Liu et al., 2011). However, studies on the degradation of carrageenan by commercial enzymes have not been reported until now.

Therefore, we were interested to find the means for the efficient production of carrageenan-derived oligosaccharides from carrageenan by hydrolysis using commercial α-amylase, which can hydrolyze the α-1,3 and/or β-1,4 glycosidic bonds in carrageenan. Thus far, very few reports have focused on the hydrolysis of carrageenan using commercial α-amylase. In the present study, the conditions for hydrolysis using α-amylase were optimized for the production process, and the product composition was examined.

2. Materials and methods

2.1. Materials

κ-Carrageenan, with molecular weight (Mw) 43×10^4 Da, was obtained from Shanghai Lanan Industries Co., Ltd (Shanghai, China). Furthermore, α-amylase, with activity of 4000 U/mg, was purchased from Fuchen Chemical reagents Co. (Tianjin, China). All other chemicals were of reagent grade.

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2.2. Hydrolyzing κ-carrageenan with α-amylase

κ-Carrageenan was dispersed in distilled water to obtain a 1% (w/v) suspension, and the pH was adjusted to 7.5 using 1 M NaOH. A 40 mg mass of α-amylase was added into a reactor containing 500 mL of κ-Carrageenan suspension, and the mixture was maintained in a thermostatic water bath at 50 °C for 4 h. Aliquots of the reaction mixture were periodically withdrawn and heated to 95 °C for 20 min to terminate the reaction.

2.3. Recovery of carrageenan-derived oligosaccharides

Aliquots of hydrolysates were filtered, concentrated to ~15% (w/v), and precipitated with 6 volumes of ethanol; the precipitates were then freeze-dried.

2.4. Analytical methods

The pH of the solutions was recorded using a digital pH meter (Model PHS-3C, CD Instruments, Shanghai, China). The ash, moisture, total sugar, and protein contents in the samples were determined according to standard methods (Hou, 2004). The reducing sugars were estimated by the method of Somogyi and expressed as the dextrose-equivalent value (Nelson, 1944).

The sugar composition in hydrolyzed carrageenan was analyzed using a Water600 double-column high-performance liquid chromatography system (LC-10A, Shimadzu, Tokyo, Japan). The first column (Sugarpark 1, 6.5 mm i.d. × 300 mm) used pure water as the mobile phase at a flow rate of 0.5 mL/min, and the column temperature was maintained at 85 °C. The second column (SpherisorbNH$_2$, 4.6 mm i.d. × 250 mm) used acetonitrile/water (70/30, v/v) as the mobile phase at a flow rate of 1 mL/min, and the column temperature was 30 °C. The detector sensitivity was 4 μRIU (micro-refractive index units), and the injected volume was 10 μL.

The intrinsic viscosity of the hydrolysates was measured according to the method described by Lai and Lii (1997). The viscosities of the κ-carrageenan and aqueous 0.3% KCl solutions were periodically measured after enzymatic degradation using an Ubbelohde capillary viscometer at 50 ± 1 °C. Then, the relative viscosity [η_r] of the degraded κ-carrageenan solution was calculated. The intrinsic viscosity [η] of the degraded κ-carrageenan was calculated according to the equation: $[\eta] = \lg \eta_r / c$ [c: concentration in g (100 mL)$^{-1}$]. We then added hot water to adjust the polysaccharide concentration to 0.5% (w/v) and allowed the solutions to settle for about 3 min before the viscosity was measured to maintain the polysaccharide molecules in the coiled conformation and to prevent intermolecular aggregation. All the measurements were carried out in six replicates.

3. Results and discussion

3.1. Effect of reaction duration on κ-carrageenan hydrolysis

Time-course studies on κ-carrageenan hydrolysis by α-amylase were carried out for a 6-h period (Fig. 1). A rapid decrease in intrinsic viscosity was observed within 3 h, a slower decrease in the range from 3 to 4 h, and no further decrease after 4 h. The optimum reaction time was thus considered 4 h. The decreased hydrolytic efficiency of α-amylase was probably due to the decreased substrate concentration, the increased product concentration, and the inactivation of α-amylase with time (Zhang, 2002).

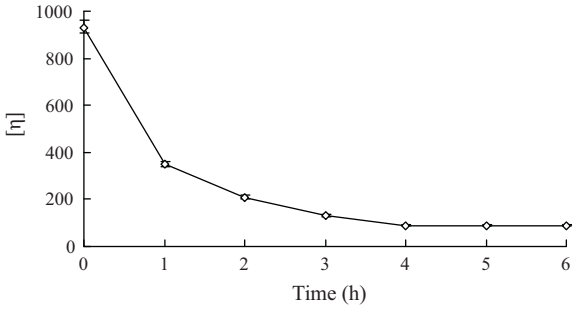

Fig. 1. Effect of time on κ-Carrageenan hydrolysis by α-amylase (the results are from three replicate experiments).

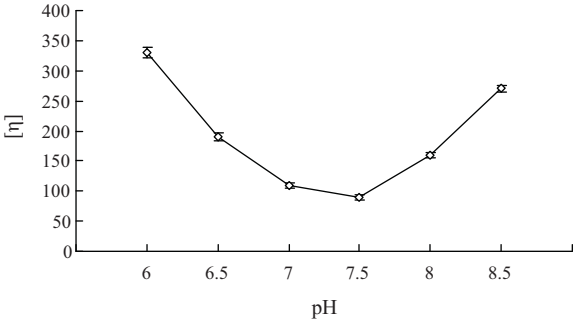

Fig. 2. Effect of pH on κ-Carrageenan hydrolysis by α-amylase (the results are from three replicate experiments).

3.2. Effect of pH, temperature, and α-amylase concentration on κ-carrageenan hydrolysis

The pH and temperature of a reaction mixture can influence α-amylase activity and, consequently, κ-carrageenan hydrolysis; moreover, α-amylase concentration may also be important for efficient hydrolysis (Zhang, 2002). Thus, the effects of different pH values, temperatures, and α-amylase concentrations on κ-carrageenan hydrolysis were investigated. The reaction was found to be optimal at pH 7.5 (Fig. 2) at a temperature of 50 °C (Fig. 3) with 40 mg α-amylase-containing reaction mixture (Fig. 4). In contrast, other reports have described varying optimal conditions for enzymatic hydrolysis of κ-carrageenan as follows: pH 5.6 (Khambhaty, Mody, & Jha, 2007), 7.5 (Zhou et al., 2008), 7.7 (Khambhaty et al., 2007), and 8.0 (Liu et al., 2011; Sun, Ma, Wang, & Liu, 2010); and temperatures of 30 °C (Sun et al., 2010; Zhou et al., 2008), 40 °C (Khambhaty et al., 2007), and 55 °C (Liu et al., 2011). These varied reports on the optimal pH values and temperatures may be due to differences in the substrates, enzyme sources, and reaction times (Zhang, 2002).

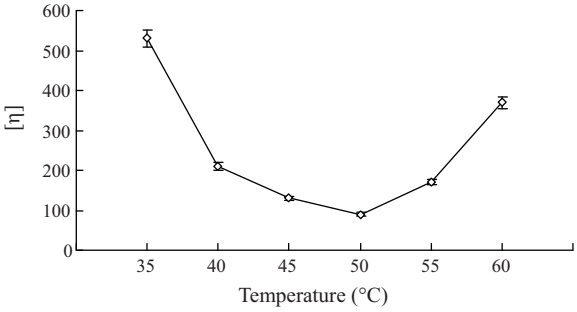

Fig. 3. Effect of temperature on κ-Carrageenan hydrolysis by α-amylase (the results are from three replicate experiments).

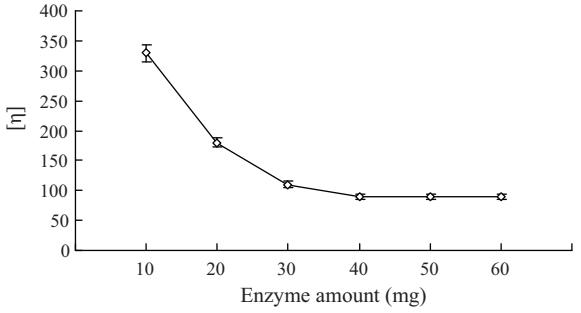

Fig. 4. Effect of enzyme amount on κ-Carrageenan hydrolysis by α-amylase (the results are from three replicate experiments).

3.3. Characterization of the product

The sugars in the hydrolyzed carrageenan were galactose (1%, w/w), (carrageenan-derived oligosaccharides)$_2$ (4%, w/w), (carrageenan-derived oligosaccharides)$_3$ (5%, w/w), (carrageenan-derived oligosaccharides)$_4$ (26%, w/w), (carrageenan-derived oligosaccharides)$_5$ (39%, w/w), (carrageenan-derived oligosaccharides)$_6$ (13%, w/w), (carrageenan-derived oligosaccharides)$_7$ (8%, w/w), and (carrageenan-derived oligosaccharides)$_8$ (4%, w/w). The ash, moisture, and total sugar contents were 0.3%, 3.2%, and 96.5% (w/w), respectively. Therefore, the purity of the carrageenan-derived oligosaccharide product was very high, with the carrageenan-derived oligosaccharides yield being 92.6% (w/w). All the product samples were white and water-soluble powders.

4. Conclusions

Carrageenan-derived oligosaccharides can be prepared by hydrolyzing carrageenan with commercial α-amylase under the following optimum conditions: pH 7.5, 50 °C, 40 mg α-amylase/500 mL of reaction mixture containing 5 g κ-Carrageenan, and 4 h, which yield products with minimum intrinsic viscosity. The hydrolysates were filtered, concentrated to ∼15% (w/v), and precipitated with 6 volumes of ethanol; the precipitates were then freeze-dried. The products were composed of κ-carrageenan-derived oligosaccharides of degree of polymerization 2–8. The κ-carrageenan-derived oligosaccharide content in the product and the κ-Carrageenan-derived oligosaccharide yield were 96.5% and 92.6% (w/w), respectively.

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