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Short communication

Preparation of xanthan-derived oligosaccharides and their hydroxyl radical scavenging activity

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ABSTRACT

In this study, the xanthan-derived oligosacchrides were prepared by hydrolysis of xanthan using hydrogen peroxide (H_2O_2) in alkaline solution. The hydrolysis process was monitored by the dextrose equivalent values of the hydrolysates. The optimal hydrolysis conditions were found to be reaction time 24 h, temperature 65 °C, H_2O_2 concentration 1.6% (v/v), and NaOH concentration, 3 M. Under these optimized conditions, the maximum dextrose equivalent value (7.53%) was observed. The structure of the hydrolysates were characterized by Fourier-transform infrared spectroscopy. The xanthan-derived oligosacchrides showed high hydroxyl radical scavenging activity. The xanthan-derived oligosacchrides content of the product and the yield were 96.8% and 95.7% (w/w), respectively.

1. Introduction

Xanthan is an exopolysaccharide produced by phyto-pathogenic bacterium *Xanthomonas campestris* pv. *campestris* (Qian, An, He, Han, & Li, 2006). Xanthan consists of a main chain of β-1,4-linked D-glucose units, as in cellulose, but with trisaccharide side chains composed of a mannosyl–glucuronyl–mannose repeating sequence attached to the C-3 position on every other glucosyl residues (Liu et al., 2005). It is a thickener with exceptional rheological properties and stability at a wide range of temperature and pH and is widely used in food industry.

Xanthan-derived oligosacchrides have elicitor, antibacterial, and antioxidant activities (Liu et al., 2005; Qian et al., 2006; Sun, Xiong, Xie, Liu, & Zhou, 2010).

They can be prepared by hydrolysis of xanthan by enzymatic, chemical, and thermal treatments (Liu et al., 2005; Qian et al., 2006; Soares, Lima, Oliveira, Pires, & Soldi, 2005; Sun et al., 2010). Hydrogen peroxide (H_2O_2) is used to hydrolyze polysaccharides such as cellulose, starch, hemicellulose, and xanthan because it is easy to handle, readily available, and environmentally friendly (Chang, Tai, & Cheng, 2001; Qin, Du, & Xiao, 2002; Shao, Yang, & Zhong, 2003; Sun et al., 2010). This technique is based on the formation of free radicals, which can attack the glucosidic linkages of the polysaccharides.

Reports on the hydrolysis of xanthan using H_2O_2 are very few. In this study, a method of preparation of xanthan-derived oligosacchrides by hydrolysis of xanthan using H_2O_2 was established. After optimizing the hydrolysis conditions, the product structure and hydroxyl radical scavenging activity (HRSA) were examined.

2. Materials and methods

2.1. Materials

Raw xanthan with a molecular weight of 1.3×10^5 Da was obtained from Zibo Zhongxuan Biochemistry Co. Ltd. (Shandong, China). H_2O_2 was purchased from the Laiyang Kant Chemical Co. Ltd. (Laiyang, China). All other chemicals were reagent grade.

2.2. Hydrolyzing xanthan with H_2O_2

Xanthan was dissolved in various concentrations of NaOH to prepare solutions with a concentration of 1% (w/v). Different volumes of H_2O_2 were added to a reactor containing 500 mL of xanthan solution and the reactor was maintained in a thermostatic water bath at different temperatures for different time. Aliquots of the reaction mixture were periodically collected and cooled below 10 °C to terminate the reaction. The dextrose equivalent (DE) values of the hydrolysates were determined to estimate the degree of the hydrolysis.

2.3. Recovery xanthan-derived oligosaccharides

The hydrolysates were filtered through Whatman GF/A filter paper, neutralized with HCl solution, concentrated to ~15% (w/v), desalted, and lyophilized to yield a white powder.

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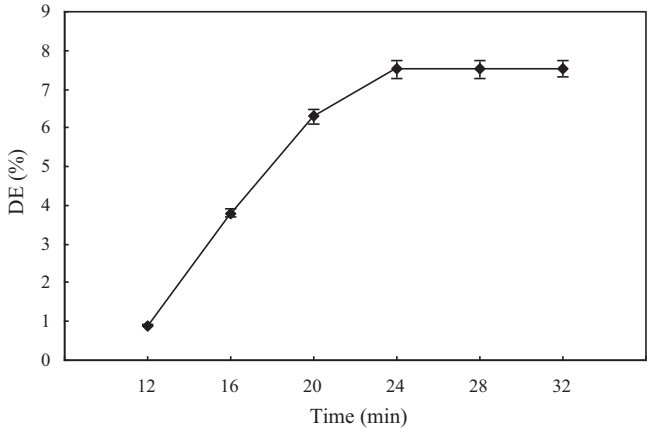

Fig. 1. Effect of time on xanthan degradation using H_2O_2. Data are shown as mean ± SD (n = 3).

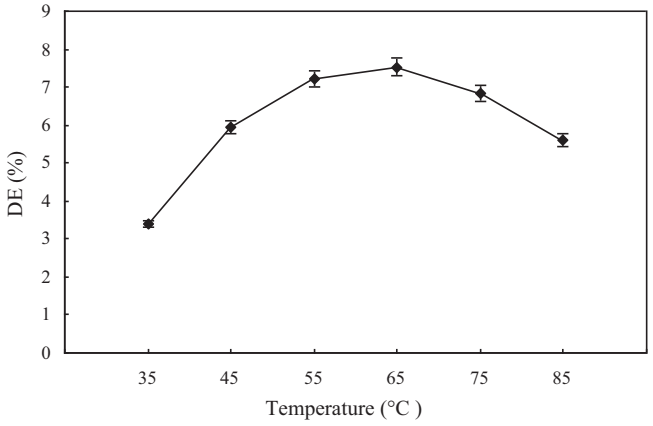

Fig. 2. Effect of temperature on xanthan degradation using H_2O_2. Data are shown as mean ± SD (n = 3).

2.4. Analytical methods

The reducing sugars were estimated by the method of Somogyi and expressed as DE value (Nelson, 1944). The DE values were used to evaluate the degree of hydrolysis of the xanthan. The Fourier-transform infrared (FTIR) spectra of representative hydrolysates samples were obtained in KBr pellets using a Nicolet Nexus FTIR 470 spectrophotometer over a wavelength range of 400–4000 cm^{-1}.

Hydroxyl radicals were generated by an iron-catalyzed Fenton Haber–Weiss reaction and the hydroxyl radicals generated were rapidly made to react with nitrone spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (Rosen & Rauckman, 1984). The resultant DMPO-OH adducts could be detectable with an electron spin resonance (ESR) spectrometer. The peptide solution (20 μL) was mixed with DMPO (0.3 mol/L, 20 μL), $FeSO_4$ (10 mmol/L, 20 μL) and H_2O_2 (10 mmol/L, 20 μL) in a phosphate buffer solution (pH 7.4), and then transferred into a 100-μL quartz capillary tube. After 2.5 min, the ESR spectrum was recorded using an ESR spectrometer. Experimental conditions for this procedure were as follows: magnetic field, 336.5 ± 5 mT; power, 1 mW; modulation frequency, 9.41 GHz; amplitude, 1 × 200; and sweep time, 4 min. HRSA was calculated according to the following equation:

$$\text{HRSA} = \frac{1 - H}{H_0 \times 100\%}$$

where H and H_0 are relative peak height of radical signals with and without sample, respectively (Qian, Jung, & Kim, 2008).

3. Results and discussion

3.1. Effect of time on xanthan hydrolysis

Reaction time is important for efficient hydrolysis. Therefore, time course studies on xanthan hydrolysis using H_2O_2 were performed for 32 h (Fig. 1). The maximum DE was obtained after 24 h. This can be rationalized on the basis of progressive consumption of H_2O_2 and xanthan with time. Therefore, the optimum hydrolysis time was 24 h.

3.2. Effect of temperature, H_2O_2 concentration, and NaOH concentration on xanthan degradation

The optimum temperature, H_2O_2 concentration, and NaOH concentration should be investigated because these parameters play pivotal roles in xanthan hydrolysis. In this study, the optimum temperature, H_2O_2 concentration, and NaOH concentration for xanthan hydrolysis were determined to be 65 °C (Fig. 2), 1.6% (v/v) (Fig. 3), and 3 M (Fig. 4), respectively. By contrast, Sun et al. (2010) observed different optimum conditions for xanthan hydrolysis: NaOH concentration of 3 mol/L and pH of 12. This discrepancy may be attributed to different xanthan sources and whether or not under microwave.

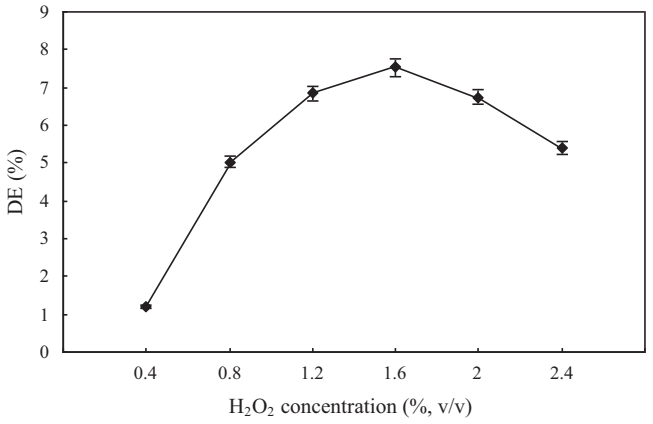

Fig. 3. Effect of H_2O_2 concentration on xanthan degradation. Data are shown as mean ± SD (n = 3).

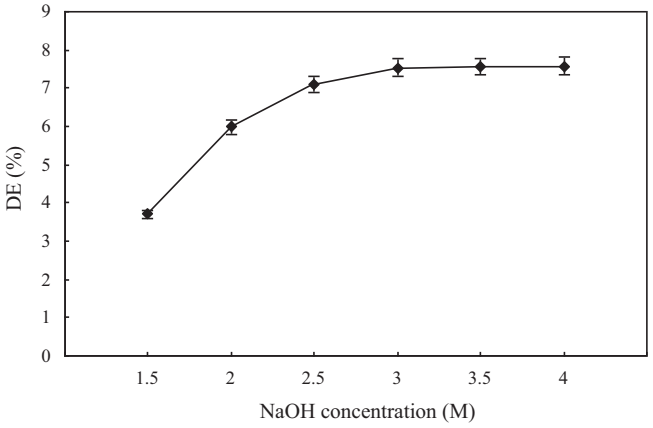

Fig. 4. Effect of NaOH concentration on xanthan degradation using H_2O_2. Data are shown as mean ± SD (n = 3).

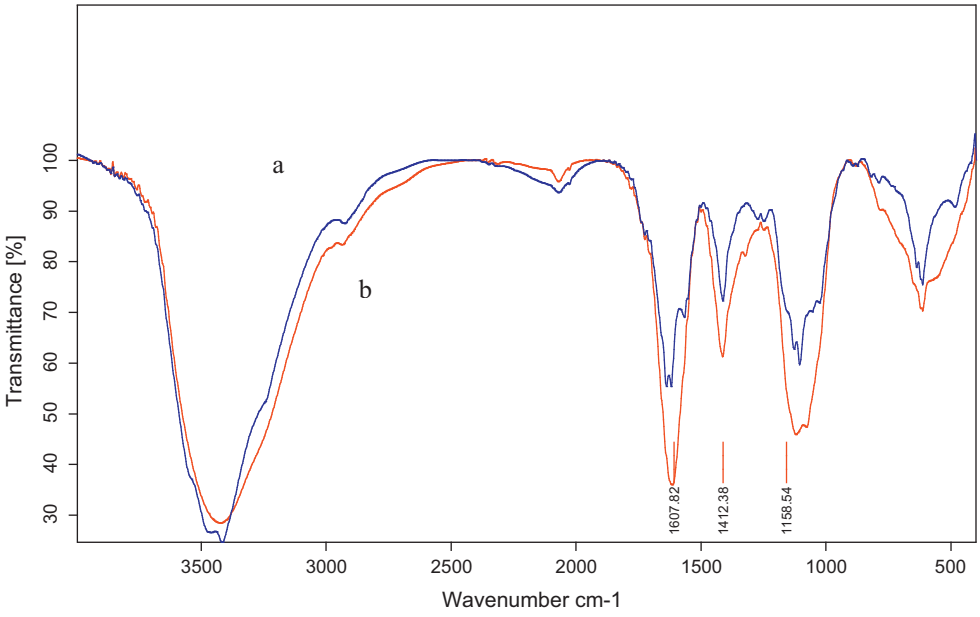

Fig. 5. FTIR spectra of original xanthan (a) and the hydrolysates (b).

3.3. Product characterization

The FTIR spectra of the original xanthan and the hydrolysates peaked at ~3500 cm^{-1} (O—H), ~1412 cm^{-1} (symmetrical deformation of —CH_3 and —CH_2), ~1607 cm^{-1} (special absorbance peaks of aldehyde in xanthan), and ~1159 cm^{-1} (special absorbance peaks of β-(1 → 4) glucoside bond in xanthan) (Fig. 5). These data demonstrated that the structures of the main chain of xanthan and the hydrolysates were the same. The DE of the resulting products was 7.54, indicating the average degree of polymerization was 13–14. The xanthan-derived oligosacchrides content of the product and the yield were 96.8% and 95.7% (w/w), respectively.

3.4. HRSA of the hydrolysates

The effect of concentration of xanthan-derived oligosacchrides on HRSA is shown in Fig. 6. The results were subjected to best-fit linear regression and the coefficients were calculated, producing a fitted equation for predicting HRSA (Y) as follows:

$$Y = 65.045 \times x - 0.0805$$

where x is the xanthan-derived oligosacchrides concentration. The regression coefficient was 0.9912 for this reaction. In general, a regression model having an R^2 value >0.9 is considered to indicate a very high correlation (Haaland, 1989). The HRSA of the xanthan-derived oligosacchrides reached 85.02% at a concentration of 2.5 mg/mL, thus indicating that the xanthan-derived oligosacchrides have a very high HRSA.

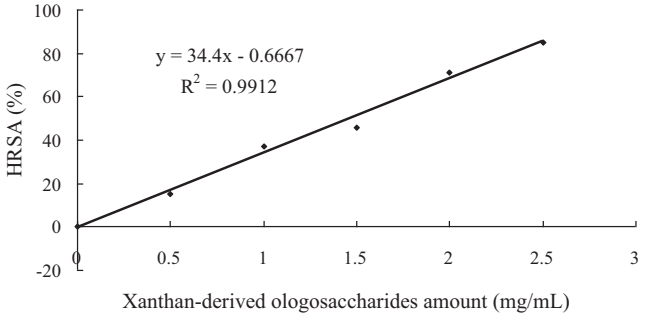

Fig. 6. The hydroxyl radical scavenging activity (HRSA) of the hydrolysates.

4. Conclusions

Xanthan-derived oligosacchrides can be prepared by hydrolysis of xanthan using H_2O_2, and the maximum DE can be obtained under the optimum conditions of 65 °C, 1.6% (v/v) H_2O_2, 3 M NaOH, and 24 h reaction time. The degree of polymerization of the xanthan-derived oligosacchrides was 13–14. The xanthan-derived oligosacchrides have a very high HRSA.

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