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Short communication

Preparation and gel properties of low molecular weight curdlan by hydrolysis of curdlan with commercial α-amylase

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ABSTRACT

Low molecular weight curdlan (LMWC) was prepared by hydrolysis of curdlan with commercial α-amylase. The hydrolysis reaction was conducted using 31.94 mg α-amylase per 500 mL reaction mixture, which contained 5 g curdlan. The hydrolysis was performed at pH 5.98 and 55.92 °C for 10 min. The molecular weight and structure of LMWC were characterized by high-performance liquid chromatography and Fourier transform infrared spectroscopy, respectively. Generally, LMWC showed lower gel strength than high molecular weight curdlan (HMWC). Unlike HMWC, LMWC could form into a gel at 50 °C. By contrast, HMWC could form into a gel at pH 11, but LMWC gel failed to form at this pH level. The strength of LMWC and HMWC gels increased with increasing temperature and decreased with increasing pH level.

1. Introduction

Curdlan, a linear glucan interconnected by β-(1 → 3) linkages, is a water-insoluble microbial exo-polysaccharide produced by a mutant strain (10C3K) of the bacterium *Alcaligenes faecalis* var. *myxogenes* 10C3. Curdlan is extensively used in the food industry because of its unique functions. In addition, modified curdlan may be applied to pharmaceutical preparations (Kanke, Koda, Koda, & Katayama,1992; Kanke, Katayama, & Nakamura, 1995; Kanke, Tanabe, Katayama, Koda, & Yoshitomi, 1995; Kim et al., 2000; Lee et al., 2001; Naito et al., 1998).

Curdlan has unique thermal gelling properties. When a curdlan suspension is heated to 60 °C and subsequently cooled, a thermally reversible gel (low-set gel) is formed. By contrast, a firm, resilient and thermally irreversible gel (high-set gel) is formed at 80 °C or at temperatures higher than 80 °C (Kanke et al., 1992). The molecular weight (M_W) of curdlan affects its liquid crystalline gel formation and aqueous suspension viscoelasticity (Nobe et al., 2005). However, the effect of curdlan MW on gel properties, including gel strength, remains undetermined.

We prepared a low molecular weight curdlan (LMWC) and investigated its gel properties. LMWC was prepared by hydrolysis of curdlan with commercial α-amylase. The resulting gel was partially characterized, and the gel strength of LMWC was examined.

2. Materials and methods

2.1. Materials

Raw curdlan, with a M_W of 37×10^4 Da, was obtained from Shanghai Lanan Industries Co., Ltd. (Shanghai, China). Commercial α-amylase, with 4000 U/mg activity, was purchased from Fuchen Chemical Reagents Co. (Tianjin, China). All other chemicals used were reagent grade.

2.2. Hydrolyzing curdlan with commercial α-amylase

Curdlan was suspended in deionized water to prepare a suspension with a concentration of 1% (w/v). Commercial α-amylase (31.94 mg) was added to 500 mL of reaction mixture containing 5 g curdlan after the suspension was heated to 80 °C and cooled to 55 °C. The reaction mixture was maintained in a thermostatic water bath at 55.92 °C and pH 5.98 for 10 min (Qian, Wu, Pan, & Xia, 2012). The resulting hydrolysates were heated to 95 °C for 20 min to terminate the reaction. After reaction termination, the hydrolysates were cooled, filtered through Whatman GF/A filter paper, neutralized with 1 M NaOH, concentrated to ~10% (w/v), desalted and freeze-dried to produce a powder.

2.3. Analytical methods

The pH of the solution was recorded using a digital pH meter (Model: PHS-3C, CD Instruments, China). Ash, moisture, total sugar,

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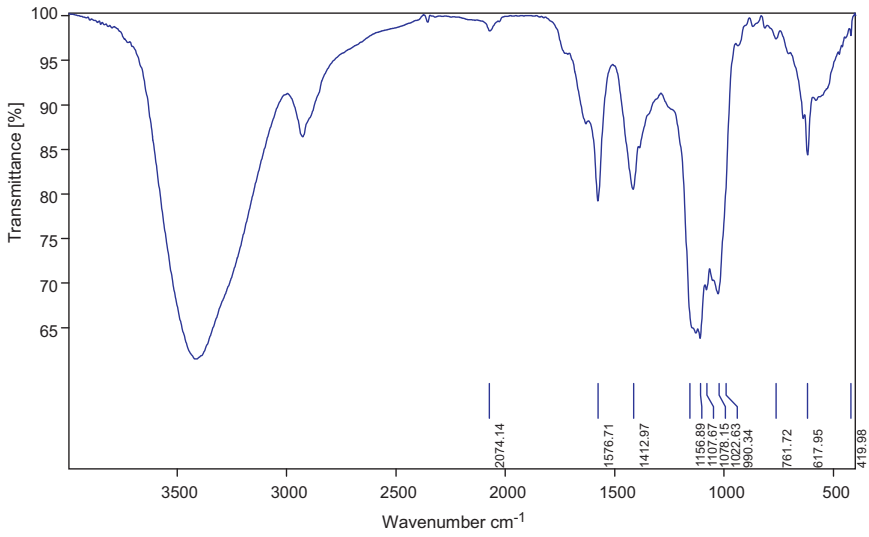

Fig. 1. FT-IR spectra of LMWC.

and protein content of the samples were determined according to standard methods (Hou, 2004). Gel strength was recorded on a texture analyzer (Brookfield, USA). Curdlan M_W was determined by high performance gel filtration chromatography on an ultrahydrogel size exclusion column, which can detect M_W in the range 10^3–10^6. In size exclusion chromatography studies, 0.3 M NaOH was used as an eluent at a flow rate of 0.9 mL/min. The detector used was a highly sensitive refractive index detector, namely, model ERC-7515A (ERC Inc., Japan). The calibration of the detector was performed using known concentrations of commercially available pullulan (Sigma, USA). An aliquot of 20 μL was injected to the column after filtration through a 0.45 μm millipore filter at ambient temperature, and the procedure was repeated thrice. The software used was the Multi-channel Chromatography Data Station (Version 144A, 1993–1997 Ampersand Ltd.). The Fourier transform infrared (FT-IR) spectra of representative hydrolysate samples were obtained in KBr pellets using a Nicolet Nexus FT-IR 470 spectrophotometer over a wavelength range of 400–4000 cm^{-1}.

Gel strength was measured on a CT3 texture analyzer (Brookfield Co., USA) with a 1.27 cm-diameter, flat-faced cylindrical plunger at a testing speed of 1 mm/s. Gel strength was expressed as the maximum force (g) obtained when the curdlan gel was compressed by 4 mm. The sample was approximately 36 mm in diameter and 25 mm in height.

2.4. Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using Statgraphics Centurion XV Version 15.1.02. A multifactor ANOVA with posterior multiple range test was used to find significant differences between the two groups.

3. Results and discussion

3.1. Characterization of the product

The ash, moisture and total sugar concentrations were 0.4, 1.9 and 97.4% (w/w), respectively. The purity of the LMWC product was very high, and the LMWC yield was 96.4% (w/w). All product samples were white powders. The M_W decreased from 37×10^4 Da to 7.3×10^4 Da, and such decrease indicated that the average degree of polymerization was ~45. The FTIR spectra of the LMWC peaked at ~3400 cm^{-1} (O–H), ~1413 cm^{-1} (symmetrical deformation of $-CH_3$ and $-CH_2$), ~1023 cm^{-1} (stretching vibration of the C–O–C in glucose circle) and ~1157 cm^{-1} (special absorbance peaks of β-(1 → 3) glucosidic bond in curdlan) (Fig. 1).

3.2. Effect of temperature on gel strength of LMWC

Temperature is critical for curdlan gel formation. The strength of LMWC gel was generally lower than that of HMWC gel, but temperatures of higher than 60 °C showed similar effects on the strength of LMWC and HMWC gels (Fig. 2). A thermally reversible gel formed in the temperature range of 60–80 °C, thermally irreversible gels formed at higher than 80 °C. The strength of thermally irreversible gel was higher than that of thermally reversible gel. Gel strength increased with temperature. LMWC could form into a gel when the temperature was lower than 60 °C, whereas HMWC could not. LMWC required high temperature to be suspended in water.

3.3. Effect of pH on gel strength of LMWC

The dissolution characteristics of curdlan are affected by pH. Curdlan failed to dissolve in neutral or acid water solution, but dissolved in alkaline water solution. Thus, curdlan gel properties, including gel strength, were affected by pH. Effects of pH on strength of both LNMWC and HMWC gels are shown in Fig. 3.

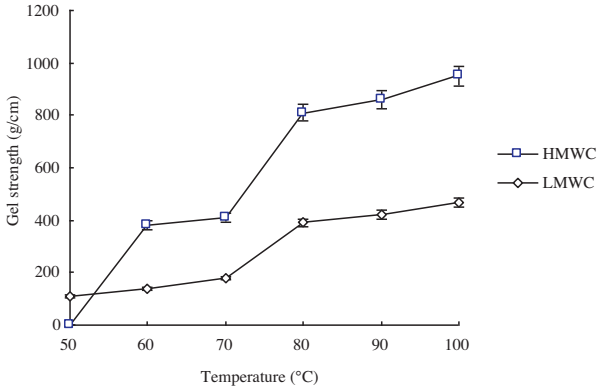

Fig. 2. Effect of temperature on gel strength of LMWC and HMWC. Bars represent the standard deviation. Data are shown as mean ± SD ($n = 3$).

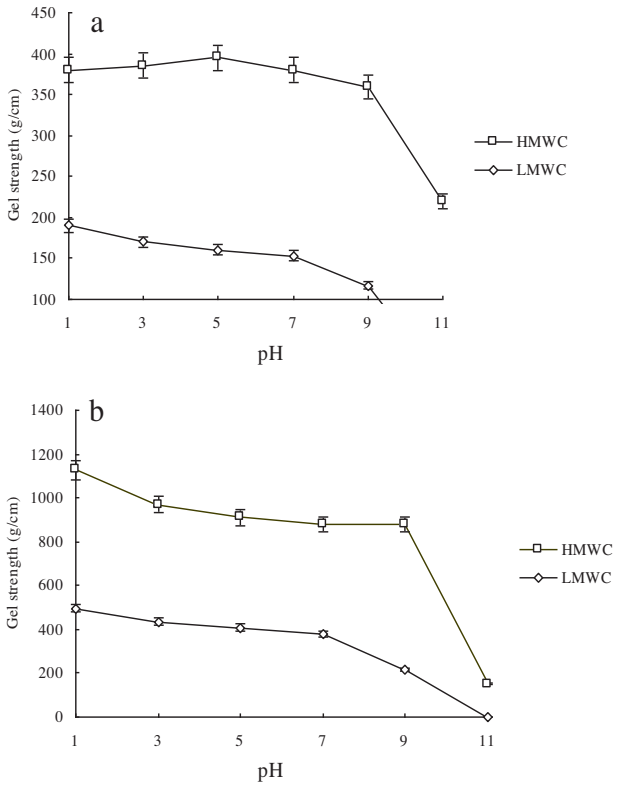

Fig. 3. Effect of pH on gel strength of LMWC and HMWC. (a) Thermally reversible gel; (b) thermally irreversible gel. Bars represent the standard deviation. Data are shown as mean ± SD ($n = 3$).

For both thermally reversible and thermally irreversible gels, the strength of both LMWC and HMWC gels steadily decreased when pH increased from 1 to 9, and abruptly decreased when pH increased from 9 to 11. LMWC failed to form either thermally reversible or thermally irreversible gels at pH 11, whereas HMWC successfully formed such gels at this pH level.

4. Conclusions

We prepared LMWC by hydrolysis of curdlan with commercial α-amylase. Reduction in LMWC M_W indicated that curdlan could be effectively degraded by commercial α-amylase. FT-IR spectra of the LMWC showed that hydrolysis with commercial α-amylase failed to damage the sugar ring in curdlan. Both thermally reversible and thermally irreversible gels showed enhanced gel strength with increasing temperature, and decreasing gel strength with increasing pH level. LMWC formed a thermally reversible gel at 50 °C, but failed to form such gel at pH 11. This finding was inconsistent with that obtained for HMWC.

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