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Short communication

Preparation of (1 → 3)-β-D-glucan oligosaccharides by hydrolysis of curdlan with commercial α-amylase

Zhigang Qian, Shengjun Wu*, Saikun Pan, Zhenqiang Xia

School of Marine Science and Technology, Huaihai Institute of Technology, 59 Cangwu Road, Lianyungang 222005, China

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ABSTRACT

Water soluble (1 → 3)-β-D-glucan oligosaccharides were prepared by hydrolyzing curdlan with α-amylase. The hydrolysis process was monitored by the DE values of the hydrolysates. Under the optimized conditions (pH, 5.98; temperature, 55.92 °C; α-amylase amount, 31.94 mg α-amylase/500 mL of reaction mixture containing 5 g curdlan; reaction time, 30 min), maximum DE value (15.62%) was obtained. The resulting products were composed of (1 → 3)-β-D-glucan oligosaccharides of DP 2-9. The hydrolysates were filtered, concentrated to ~20% (w/v), and precipitated with 5 volumes of ethanol, which were then freeze dried to yield a water soluble powder. The (1 → 3)-β-D-glucan oligosaccharides content of the product and the yield were 97.7% and 97.6% (w/w), respectively.

1. Introduction

Curdlan, a polysaccharide produced by a mutant strain (10C3K) of the bacterium, *Alcaligenes faecalis* var. *myxogenes* 10C3, is a linear glucan interconnected by β-(1 → 3) linkages. This homopolysaccharide is water insoluble but dissolves in alkaline solutions. Due to its unique functions including its thermal gelling properties, curdlan is widely used in food industry. Moreover, modified curdlan may be applied to pharmaceutical preparations (Kanke, Katayama, & Nakamura, 1995; Kanke, Koda, Koda, & Katayama, 1992; Kanke, Tanabe, Katayama, Koda, & Yoshitomi, 1995; Kim et al., 2000; Lee et al., 2001; Naito et al., 1998).

By analogy with the fructo- and xylooligosaccharides (both described as prebiotics), hypotheses have been made that (1 → 3)-β-D-glucan oligosaccharides from curdlan could also present prebiotic properties and would therefore be a potentially new ingredient for the nutraceutical market (Grandpierre, Janssen, Laroche, Michaud, & Warrand, 2008). In order to further confirm the prebiotic activity of (1 → 3)-β-D-glucan oligosaccharides, it is necessary to prepare them in large quantities. Degradation of the curdlan can be realized by either enzymic or acidic treatment (Grandpierre et al., 2008).

Therefore, it was of our interest to find means for efficient production of (1 → 3)-β-D-glucan oligosaccharides from curdlan by hydrolysis using commercial α-amylase, which can hydrolyze the β-(1 → 3) glycosidic bonds in curdlan. So far, very few reports have focused on hydrolysis of curdlan using commercial α-amylase. In the present study, the α-amylase hydrolysis conditions were optimized for the production process and product composition was examined.

2. Materials and methods

2.1. Materials

Raw curdlan, with molecular weight (Mw) 37×10^4 Da, was obtained from Shanghai Lanan Industries Co., Ltd. (Shanghai, China). α-Amylase, with 4000 U/mg activity, was purchased from Fuchen Chemical reagents Co. (Tianjin, China). All other chemicals were reagent grade.

2.2. Hydrolyzing curdlan with α-amylase

Curdlan was dispersed in distilled water to make a suspension of a concentration of 1% (w/v) and the pH adjusted to 5.98 using 1 M HCl. A 31.94 mg mass of α-amylase was added into a reactor containing 500 mL of curdlan suspension and then maintained in a thermostatic water bath at 55.92 °C for 30 min. Aliquots of reaction mixture were periodically withdrawn and heated to 95 °C for 20 min to terminate the reaction.

2.3. Recovery of (1 → 3)-β-D-glucan oligosaccharides

Aliquots of hydrolysates were filtered, concentrated to ~20% (w/v), and precipitated with 5 volumes of ethanol, which were then freeze dried.

* Corresponding author. Tel.: +86 0518 8589547; fax: +86 0518 85895428.
E-mail address: wushengjun008@sina.com (S. Wu).

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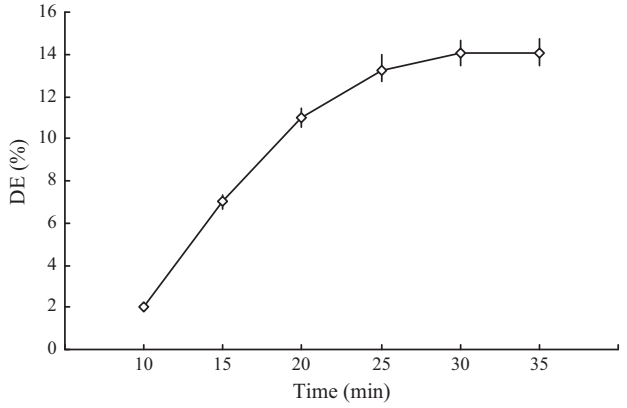

Fig. 1. Effect of time on curdlan hydrolysis by α-amylase (the results are from three replicate experiments).

2.4. Analytical methods

The pH of the solution was recorded using a digital pH meter (Model: PHS-3C, CD Instruments, China). Ash, moisture, total sugar, and protein content of the samples were determined according to standard methods (Hou, 2004). The reducing sugars were estimated by the method of Somogyi and expressed by dextrose equivalent (DE) value (Nelson, 1944). DE values were used as an index of the enzyme activities in the reaction mixture. The composition of the sugar in hydrolyzed curdlan was analyzed by Water600 HPLC equipped with a double column system (LC-10A, Shimadzu, Japan). The first column (Sugarpark 1, 6.5 mm id × 300 mm) used pure water as mobile phase at a flow rate of 0.5 mL/min and the column temperature was maintained at 85 °C. The second column (Spherisorb NH_2, 4.6 mm id × 250 mm) used acetonitrile/water (70/30, v/v) as mobile phase at a flow rate of 1 mL/min and the column temperature was 30 °C. The detector sensitivity was 4 μRIU and the inject volume was 10 μL.

2.5. Experimental design

A Central-Composite Design (CCD) was used to optimize the hydrolysis conditions. The Design Expert (Version 7. 1. 6, State-Ease Inc., Minneapolis, USA) software was used for the experimental design, data analysis, and model building.

3. Results and discussion

3.1. Effect of time on curdlan hydrolysis

Reaction time affects the α-amylase activity and is important for efficient hydrolysis. Therefore, time course studies on curdlan hydrolysis by α-amylase were performed for a 35 min period (Fig. 1). There was a rapid increase in DE within 25 min, a slower increase from 25–30 min, and no further increase after 30 min. Therefore, the optimum hydrolyzing time was judged to be 30 min.

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

A reaction mixture's temperature, pH, and the α-amylase concentration, which play pivotal roles in curdlan hydrolysis, were further investigated for their optimum combination using a CCD. The design and results of the experiments conducted using a CCD are shown in Table 1. Results were analyzed using ANOVA, and the

Table 1
The Central-Composite Design for optimizing hydrolysis conditions.

Run	Parameters						DE
	Coded levels			Actual levels			
	X_1	X_2	X_3	X_1	X_2	X_3	
1	0	−1.68	0	55	5.16	28	7.39
2	0	0	−1.68	55	6.00	21.27	8.18
3	+1.68	0	0	63.41	6.00	28	10.59
4	+1	+1	+1	60	6.50	32	10.82
5	−1.68	0	0	46.59	6.00	28	8.06
6	−1	+1	+1	50	6.50	32	8.69
7	+1	0	−1	60	5.50	24	7.46
8	0	0	0	55	6.00	28	15.01
9	−1	0	−1	50	5.50	24	6.31
10	0	+1.68	0	55	6.84	28	8.01
11	−1	0	+1	50	5.50	32	11.76
12	+1	+1	−1	60	6.50	24	8.10
13	0	0	0	55	6.00	28	14.21
14	0	0	+1.68	55	6.00	34.73	14.76
15	+1	0	+1	60	5.50	32	11.35
16	−1	+1	−1	50	6.50	24	6.55
17	0	0	0	55	6.00	28	14.89
18	0	0	+1.68	55	6.00	34.73	15.71
19	0	0	0	55	6.00	28	14.86
20	0	0	0	55	6.00	28	15.31

X_1, temperature (°C); X_2, pH; X_3, enzyme amount (mg).

regression model was obtained as follows:

$$Y = -692.61381 + 8.42507X_1 + 125.36133X_2 + 6.43243X_3 + 0.14700X_1 \times X_2 - 0.0061250X_1 \times X_3 - 0.28000X_2 \times X_3 - 0.081896X_{12} - 10.48766X_{22} - 0.070068X_{32}$$

where Y is the DE values (%), X_1 is the temperature (°C), X_2 is the pH of the mixture, and X_3 is the α-amylase concentration. The results are summarized in Table 2. As demonstrated by the F-value and the probability value [$(P > F) < 0.0001$], the model was highly significant. The accuracy of fit was proven by the high multiple correlation coefficient ($R^2 = 98.32\%$), and the value of the adjusted multiple correlation coefficient ($R^2_{Adj} = 96.81\%$) was also sufficiently high to indicate the significance of the model (Haaland, 1989).

The interaction between temperature and pH and that between temperature and α-amylase concentration are not significant, while that between pH and α-amylase concentration is significant (Table 2). The optimal conditions for the maximum DE based on the model were calculated as 55.92 °C, 5.98, and 31.94 mg α-amylase/500 mL of reaction mixture containing 5 g curdlan for temperature, pH, and α-amylase concentration, respectively. By substituting levels of the factors into the regression equation, the maximum predictable response for DE was calculated and

Table 2
Analysis of variance for the experimental results of the Central-Composite Design.

Factor	Sum of square	Degree of freedom	F value	$P > F$	Significance
X_1	5.51	1	15.03	0.0031	**
X_2	0.21	1	0.56	0.4708	
X_3	52.84	1	144.13	<0.0001	**
X_1^2	60.41	1	164.76	<0.0001	**
X_2^2	99.07	1	270.21	<0.0001	**
X_3^2	18.11	1	49.40	<0.0001	**
$X_1 \times X_2$	1.08	1	2.95	0.1168	
$X_1 \times X_3$	0.12	1	0.33	0.5798	
$X_2 \times X_3$	2.51	1	6.84	0.0258	*
Model	215.00	9	65.15	<0.0001	**
Lack of fit	3.01	1	0.60	4.59	
Pure error	0.66	5			

* Statistically significant at 95% of probability level.
** Statistically significant at 99% of probability level.

was experimentally verified. The maximum DE obtained experimentally using the optimized medium was 15.62%, which was consistent with the predicted value of 15.75% obtained using a response surface methodology regression study.

3.3. Characterization of the product

The sugars in the hydrolyzed curdlan were glucose (1%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_2$ (3%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_3$ (8%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_4$ (21%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_5$ (32%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_6$ (17%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_7$ (9%, w/w), $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_8$ (6%, w/w), and $((1\rightarrow3)$-β-D-glucan oligosaccharides$)_9$ (3%, w/w), respectively. The ash, moisture, and total sugar were 0.2, 2.1, and 97.7% (w/w), respectively. Therefore, the purity of the $(1\rightarrow3)$-β-D-glucan oligosaccharides product was very high, with the $(1\rightarrow3)$-β-D-glucan oligosaccharides yield at 97.6% (w/w). All product samples were white powders and water soluble.

4. Conclusions

$(1\rightarrow3)$-β-D-Glucan oligosaccharides can be prepared by hydrolyzing curdlan with commercial α-amylase under optimum conditions of pH 5.98, 55.92 °C, 31.94 mg α-amylase/500 mL of reaction mixture containing 5 g curdlan, and 30 min, affording the maximum DE. The hydrolysates were filtered, concentrated to ~20% (w/v), and precipitated with 5 volume of ethanol, which were then freeze dried. The products were composed of $(1\rightarrow3)$-β-D-glucan oligosaccharides of DP 2-9. The $(1\rightarrow3)$-β-D-glucan oligosaccharides content in the product and the $(1\rightarrow3)$-β-D-glucan oligosaccharides yield were 97.7% and 97.6% (w/w), respectively.

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