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Short communication

Determination via hydrolysis with α-amylase of curdlan in the presence of proteins

Zhigang Qian, Saikun Pan, Shengjun Wu*

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

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ABSTRACT

A novel method of determining curdlan in the presence of water-insoluble but alkaline solution-soluble proteins via hydrolysis with α-amylase was developed. The hydrolyzed products were mainly composed of the reducing sugars (1→3)-β-D-glucan oligosaccharides, which were determined using the 3,5-dimethylsalicylic acid method. The observed absorbance at 540 nm was linear with respect to the curdlan concentration (regression coefficient: 0.9897). The new method is specific for the determination of curdlan and exhibits high accuracy, particularly in the sample containing water-insoluble but alkaline solution-soluble proteins. The method can be used to assay the curdlan content up to 3.2 mg/mL and can overcome the shortcomings of the gravimetric method.

1. Introduction

Curdlan, a linear glucan interconnected by β-(1→3) linkages, is a homopolysaccharide produced by a mutant strain (10C3K) of the bacterium, *Alcaligenes faecalis* var. myxogenes 10C3. Curdlan is widely used in the food industry because of its unique functions, including its thermal gelling properties (McIntosh, Stone, & Stanisich, 2005).

Curdlan has usually been gravimetrically determined based on its water-insoluble, alkaline solution-soluble nature (Lee & Park, 2001; Shih, Yu, Hsieh, & Wu, 2009; Yu et al., 2011). However, the gravimetric method is not applicable for the determination of curdlan in the presence of other substances with a similar nature, including many proteins.

Based on our previous studies (Qian, Wu, Pan, & Xia, 2011), α-amylase is capable of hydrolyzing β-(1→3) glycosidic bonds in curdlan to produce (1→3)-β-D-glucan oligosaccharides. These hydrolysis products are reducing sugars because of the presence of the reducing hemiacetal hydroxyl group at one end. Therefore, they can reduce 3,5-dimethylsalicylic acid, and the reaction can be colorimetrically determined. Thus, a novel method for the determination of curdlan was developed in the current study. This method could overcome the shortcomings of the gravimetric method.

2. Materials and methods

2.1. Materials

Standard curdlan (99.9%, w/w) was obtained from Sigma–Aldrich (USA). Curdlan 1 (96%, w/w) was obtained from Takeda Co., Ltd (USA). α-amylase, with 4000 U/mg activity, was purchased from Fuchen Chemical Reagents Co. (Tianjin, China). Casein (99%, w/w) was purchased from Henan Province Keyi Chemical Co., Ltd (China). Bovine serum albumin (BSA) was obtained from Sigma–Aldrich (USA). All other chemicals were of reagent grade.

2.2. Color reagent

Reagent A was prepared by dissolving phenol (6.9 g) in 15.2 mL 2.5 M NaOH. Sodium sulfite (6.9 g) in distilled water (70 mL) was subsequently added to the solution. Reagent B was prepared by dissolving potassium sodium tartrate (255 g) in 300 mL 2.5 M NaOH. 3,5-dimethylsalicylic acid (10 g/L, 880 mL) was then added to the solution. The color reagent was prepared by mixing Reagent A with Reagent B, and the resulting solution was stored in a brown bottle at 25 °C for 10 d (Wu, Kim, Zhou, Jin, & Tong, 2010).

2.3. Assay

Curdlan was dispersed in distilled water to form a suspension with a concentration of 0.2% (w/v). The pH was then adjusted to 5.98 using 1 M HCl. α-amylase (31.94 mg) was added into a reactor containing 500 mL of the curdlan suspension, which was then

* Corresponding author. Tel.: +86 518 85895427; fax: +86 510 85895428.
E-mail address: wushengjun008@sina.com (S. Wu).

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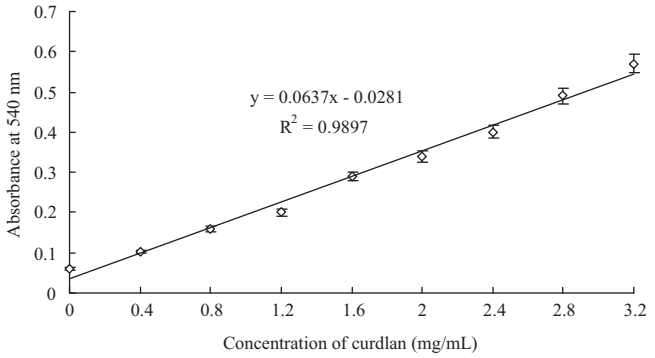

Fig. 1. Plot of the relative absorbance versus the curdlan concentration. Data are shown as the mean ± S.D ($n = 6$).

maintained in a thermostatic water bath at 55.92 °C for 30 min (Qian, Pan, Xia, & Wu, 2011).

Different volumes of hydrolysate (up to 1.6 mL) were added to a series of test tubes, and the volumes were adjusted to 2.0 mL using distilled water. The color reagent (1.5 mL) was then added into the test tubes, with thorough mixing. The solutions were held at 100 °C for 5 min, cooled to 20 °C, adjusted to 25 mL with distilled water, and filtered. The absorbance of a 1 mL hydrolysate sample was determined at 540 nm. Casein, BSA, and samples 1, 2, and 3 were assayed using the same method. The curdlan contents of the same samples were determined via the gravimetric method (Lee & Park, 2001).

2.4. Statistical analysis

All data are presented as mean ± S.D. A student's *t*-test was used to compare the means of two groups. Relationships were analyzed using Pearson's correlation coefficient *R*. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Standard curve

The standard curve for the curdlan concentration is shown in Fig. 1. The data were subjected to a good-fit linear regression and the coefficients were calculated, producing a fitted equation for predicting the curdlan content (X), as follows:

$$Y = 0.0637 \times X - 0.0281$$

where Y is the absorbance.

The regression coefficient is 0.9897. On the other hand, an R^2 value of a regression model higher than 0.9 is generally considered a very high correlation (Haaland, 1989). Therefore, the concentration of curdlan in the samples can be calculated according to the absorbance value and using the above equation.

3.2. Curdlan content of the different samples

The curdlan contents of the samples are shown in Table 1. For curdlan sample 1, the curdlan contents determined via the α-amylase method are comparable with those determined by the gravimetric method, indicating that curdlan was the main sugar form in this sample. For the casein samples, which were water-insoluble but alkaline solution-soluble, the results were significantly dependent on the assay method used ($P < 0.0001$). The curdlan content determined by the gravimetric method was erroneously high (71.3%, w/w), whereas the content determined using α-amylase was zero, indicating that the α-amylase method is capable of differentiating between curdlan and water-insoluble, alkaline solution-soluble proteins. Hence, this method is suitable for assaying the curdlan content in samples containing the protein. For the BSA samples, which were both water- and alkaline solution-soluble, the curdlan content determined by either the α-amylase method or the gravimetric method was zero, indicating that both methods are capable of differentiating between curdlan and water- and alkaline solution-soluble proteins.

Table 1
Curdlan contents of chitosan 1, casein, and BSA determined by α-amylase method and gravimetric method.

Sample	α-amylase (%, w/w)[a]	Gravimetric (%, w/w)[a]
Curdlan 1	95.4 ± 1.1	96.1 ± 1.2
Casein	0	71.3 ± 1.3
BSA	0	0

[a] Mean ± S.D. from three independent experiments.

4. Conclusions

The present results indicate that the α-amylase method is a simple, rapid, and reliable means of determining the curdlan content in samples containing water-insoluble but alkaline solution-soluble proteins. Furthermore, it is cost-effective and does not require relatively expensive equipment and analytical materials.

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