

Production of Red Bean Starch Granule with Cellulase from *Fusarium moniliforme*

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Fusarium moniliforme 이 생산하는 셀룰라아제를 이용한 小豆澱粉製造

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요 약

Fusarium moniliforme 액체배양으로 부터 얻어진 조효소를 황산암모늄 분획침전, Sephadex G-25, Sephadex G-75, Sephadex G-150 및 DEAE-Sephadex A-50 컬럼크로마토그래피를 통하여 2개의 여지분해 효소와 2개의 β -glucanase를 분리하고 팔전분 제조에 이용하였다. 팔을 50°C에서 2시간동안 섬유소 분해효소와 작용시킨 결과 효소처리구는 세포벽, 세포간극 그리고 전분입자간극이 일부 분해되었음을 알 수 있었다. 0.004 units/ml의 여지분해효소와 0.3 units/ml의 β -glucanase를 혼합 처리했을 때 팔전분 입자의 침강속도가 최대가 되었고 0.004 units/ml의 여지분해효소와 0.2 units/ml의 β -glucanase를 혼합하여 처리했을 때 수율증가는 약 7%이었다. 증자후와 마쇄후의 폐수에서 혼탁물질은 효소처리구가 대조구보다 약 40% 정도 감소되었다.

Introduction

The cellulase system comprises mainly three classes of enzymes(1) : 1,4- β -D-glucan cellobiohydrolase(EC 3.2.1.91; C₁), 1,4- β -D-glucan glucohydrolase(EC 3.2.1.4; C_x) and β -glucosidase(EC 3.2.1.21). C₁ causes both disruption of cellulose hydrogen bonding and hydrolysis of cellulose chains. Endo-C_x attacks at random the β -1, 4-linkages along the cellulose chain

and exo-C_x splits off cellobiose or glucose units from the nonreducing end of the cellulose. β -glucosidase hydrolyses cellobiose and water-soluble cellodextrins to glucose.

Many have tried to develop a broad scope application of cellulase for food processing (2~4) : (a) starch isolation from potato; (b) clarifying of fruit juice; (c) brewing; (d) production of soybean curd; and others. Red bean is mainly used as a main ingredient in Oriental style cake and pie fillings, bean jam,

bread making and confectionery industry in Asian countries(5, 6).

Red bean cell comprises from 3 to 7 starch granules and starch granule is surrounded by protein membrane(7). Intercellular space and interstarchgranular space are filled with cellulose primarily. If red bean is submerged with cellulase, the cell wall, intercellular space and interstarchgranular space are partially hydrolysed by cellulase and then it absorbs water more rapidly. Consequently, most starch granules can be collected intact without being broken during boiling and maceration.

In this paper, cellulase was partially purified from *Fusarium moniliforme* and for improving the recovery of the starch granule the action pattern of cellulase to intercellular space of red bean was investigated.

Materials and Methods

Enzyme purification

Fusarium moniliforme was obtained from the Department of Agricultural Biology, Seoul National University. Red bean was purchased from the local market. *Fusarium moniliforme* was grown on wheat bran media by submerged culture for 7 days at 30°C(8). Culture broth was purified through ammonium sulfate fractionation, gel permeation and ion-exchange chromatography, as shown in Fig. 1.

Enzyme assay

Carboxymethyl cellulose (CMC) saccharifying activity (CMCase). 0.5ml of 1% solution of carboxymethyl cellulose in 0.05M citrate buffer

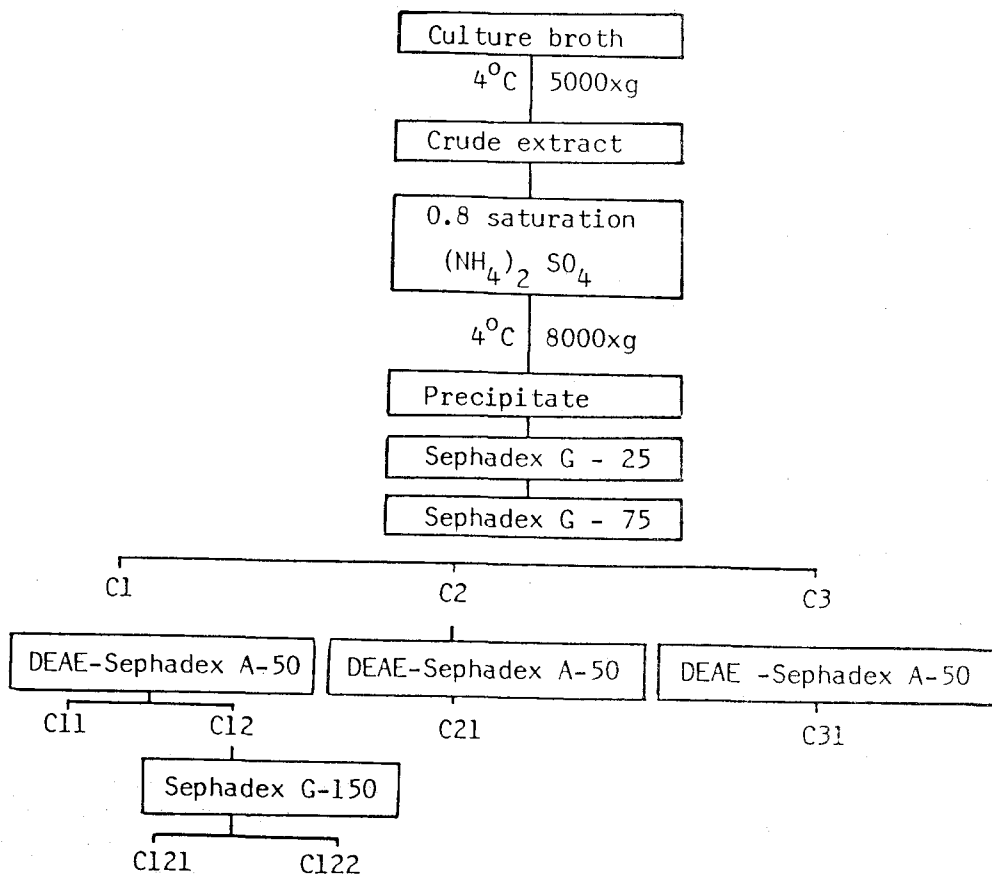


Fig. 1. Purification diagram of cellulase from *Fusarium moniliforme*

pH 5.0 was incubated with 0.5ml of enzyme solution for 30 minutes at 50°C. Reducing sugar was determined with dinitrosalicylic acid (9). Enzyme unit was expressed in μmol of reducing sugar (glucose equivalents) produced per minute.

CMC liquefying activity. One ml of the enzyme solution was added to 10ml of 1% sodium carboxymethyl cellulose in Ostwald viscometer, incubated at 50°C for 10 minutes and the flow time of the mixture was determined. The unit of enzyme activity was expressed as percent viscosity change.

Filter paper degradation activity. Activity toward filter paper was assayed according to Mendels et al(10). Two strips (1×3cm, 25mg) of Whatman No. 1 filter paper, 1.5ml of buffer and 1.0ml of enzyme solution were incubated for 3 hours at 50°C and reducing sugar was determined. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μ mole of equivalent glucose per minute.

β -Glucosidase activity. 0.5ml of enzyme solution was added to 0.5ml of 1% salicinsolution, incubated at 50°C for 30 minutes and 3ml of dinitrosalicylic acid reagent was added to mixture for determination of reducing sugar. One unit of β -glucosidase was defined as 1 μ mol of glucose equivalents released per minutes.

Preparation of red bean starch precipitate and determination of sedimentation rate. 20g of red bean was submerged in water of enzyme solution at 50°C for 2 hours and boiled immediately for 75 minutes. The supernatant was poured off, the precipitate was washed with 500ml water, macerated with spoon and filtered through cheese gauze. Red bean starch precipitate was washed with 200ml water again. 40ml of red bean starch precipitate suspension was inserted into 100ml mess cylinder and the rest volume was filled with water. The supernatant volume was determined in the course of time immediately after shaking throughly.

Determination of percent recovery of red bean starch precipitate and suspended solid.

Red bean starch precipitate filtered through gauze was dried for 72 hours at 90°C in a dry oven and then its weight was determined. Suspended solid was expressed as the difference between total solids of solution and soluble solids of solution.

Sample preparation for microscopic examination. Red bean sections, 2~3cm thick, were

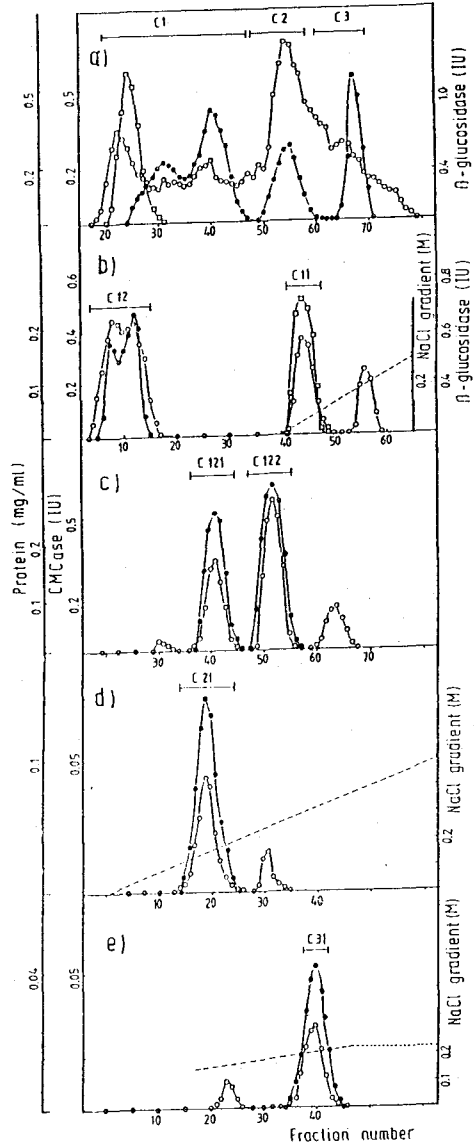


Fig. 2. Elution profiles from Sephadex G-75 (a), DEAE-Sephadex A-50(b, d and e) and Sephadex G-150 chromatographic columns (c) ○—○ protein : ●—● CMCcase : □—□ β -glucosidase

fixed in FAA(formalin-acetic-alcohol) for 24 hours, dehydrated in a normal butyl alcohol series and embedded in paraffin. Cross sections, 20mm thick, were made with a rotary microtome. Sections were stained with iron-hematoxylin.

Results and Discussion

Enzyme purification

As can be seen in Fig. 2, one peak of β -glucosidase and 4 peaks of CMCase were obtained from Sephadex G-75 column. The fractions of C1, C2 and C3 were pooled, concentrated and applied to a column of DEAE-Sephadex A-50, respectively. β -glucosidase fraction was separated from CMCase fraction (Fig. 2, b). C21 fraction was eluted at 0.15M NaCl(Fig. 2, c). C31 was eluted at 0.2M NaCl from C3 fraction(Fig. 2, e). Portion C12 was applied to Sephadex G-150 column, and two peaks of C121 and C122 were obtained(Fig. 2, b and c).

CMC saccharifying activity (CMCase) and filter paper degradation activity (FPase)

The properties of enzyme fractions toward substrates were assayed and shown in Table 1. C121 showed high CMC saccharifying and liquefying activity, but no activity on filter paper. This indicates that C121 hydrolyzes cellulose chains randomly and C121 may be β -1,4 D-glucan glucanase (endo-glucanase). C21 contained low CMC saccharifying activity, and filter paper degradation activity. On the

other hand, C122 and C31 showed high filter paper degradation activity and high CMC saccharifying activity. It is supposed that C122 and C31 can be β -1, 4 glucan cellobiohydrolase.

Effect of CMCase and FPase on sedimentation rate

Fraction C121 (CMCase) and C31 (FPase) were applied to red bean to characterize the enzyme action pattern. As can be seen in Fig. 3 sedimentation rate of red bean starch increased significantly in the range of FPase concentration of 0.001~0.006 units/ml(Fig. 3). Compared with CMCase, FPase had more effect

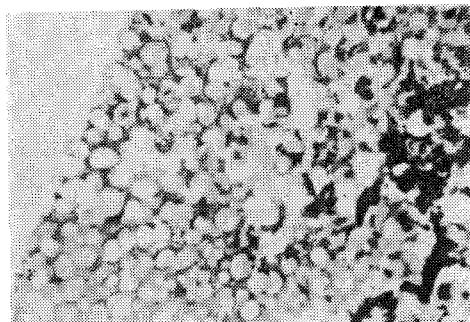
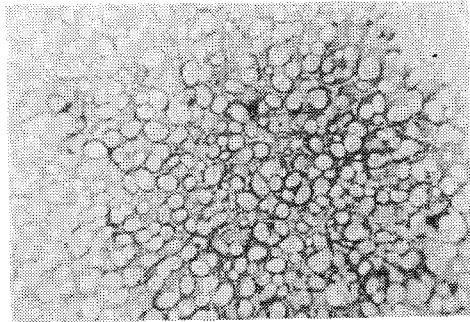


Fig. 3. Effect of cellulase on sedimentation of red bean starch a) CMCase b) FPase

Table 1. Activities of cellulase fraction

Fraction	Specific activity	CMC saccharifying activity (μ mol glucose/min)	Filter paper degradation activity (μ mol glucose/min)	CMC liquefying activity (%)
C121		3.04	0	2688
C122		2.2	0.563	137.5
C 21		0.52	0.01	482.3
C 31		2.57	0.746	117.6

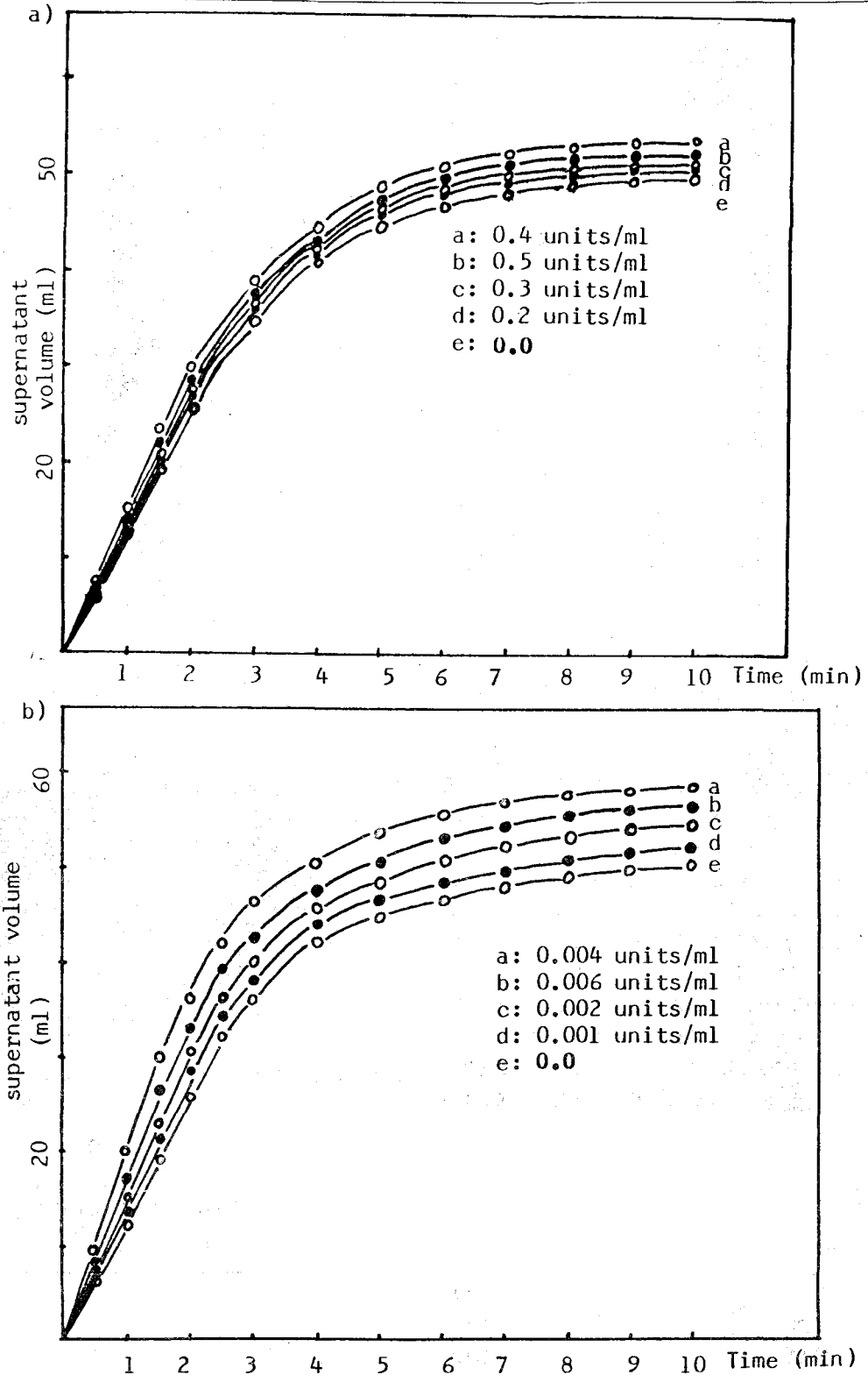


Fig. 4. Fotomicrographs of red bean tissue a) submersion in water($\times 100$) b) submersion in cellulase solution ($\times 100$)

Table 2. Effect of cellulase on recovery of red bean starch

Enzyme mixture of FPase and CMCase (IU/ml)	Red bean starch precipitate (dry weight, gram)*	% Increase
0.004 : 0.2	11.975	7.01
0.004 : 0.1	11.935	6.66
0.004 : 0.3	11.915	6.48
0.004 : 0.36	11.831	5.73
0.004 : 0.05	11.713	4.76
0.004 : 0.5	11.66	4.20
0.004 : 0.04	11.59	3.57
0 : 0	11.19	0

* All data were derived from 20g sample and values are mean of three trials.

Table 3. Suspended solid in waste water from red bean recovery process

Treatment	Suspended solid (mg)	
	after boiling	after maceration
Control	2256.94	5633.50
Cellulase mixture	1300.02	3569.20

* All data were derived from 100g sample and values are mean of two trials.

on sedimentation rate of red bean starch.

Cross sections of red bean after submersion in water and enzyme solution were shown in Fig. 4. When red bean was treated with cellulase fractions, there was a little hydrolysis in cell wall, intercellular space and interstarchgranular space. Watanabe(7) reported that starch granules in red bean are swollen, but not solubilized. We suppose that red bean treated with enzyme absorbed more water and specific gravity of starch granule increased and consequently sedimentation rate increased.

Recovery of red bean starch precipitate

Maximum percent recovery of red bean starch precipitate was obtained (Table 2), when the red bean was incubated with the mixture of 0.004 units/ml of FPase and 0.2 units/ml of CMCase. This can be explained that intercellular space and interstarchgranular space of red bean is suitably hydrolyzed by CMCase and FPase, and therefore starch granules could

be collected unbrokenly in the process of boiling and maceration. Aisawa (5) observed a significant increase in recovery of red bean starch by using commercial cellulase from *Fusarium moniliforme*.

Suspended solid in waste water after boiling and maceration

As shown in Table 3, soluble solid in waste water decreased about 40% in the enzyme treated process. The result indicates that the level of waste water pollution can be reduced by using cellulase in red bean starch processing.

Abstract

Two fractions of β -glucanase (CMCase), two fractions of filter paper degradation enzyme (FPase) and one β -glucosidase fraction were partially purified from *Fusarium moniliforme* and applied to recovery process of red bean starch.

Red bean were incubated with the fractions

of CMCase and FPase at 50°C for 2 hours and the starch granules are separated. Maximal sedimentation rate of red bean starch granules was obtained with treatment of the mixture solution of 0.004 units/ml of FPase and 0.3 units/ml of CMCase. In the enzyme treated process percent recovery of red bean starch granule increased about 7% and suspended solid in waste water was reduced about 40%, compared with those of control.

The results indicated that red bean cell treated with cellulase fractions absorbed water more rapidly and specific gravity of starch granule increased.

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