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PREPARATION OF UNBRANCHED GLUCANS BY HYDROLYSIS OF STARCH OF VARIOUS BOTANICAL ORIGIN WITH PULLULANASE

Maciej Kujawski, Rafał Ziobro, Halina Gambuś
Faculty of Food Technology, Cracow Agricultural University, Cracow, Poland

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ABSTRACT

The use of pullulanase preparation was checked as a method to obtain long unbranched glucans from native starch. Different starch sources as well as various solubilisation procedures were tried in order to choose the optimal one. Dissolving in NaOH proved to be suitable for all starch sources, while autoclave should be avoided in case of cereal starches. Potato starch gave clear solutions irrespective of solubilisation method, which were highly susceptible to enzymatic attack. Basing on the results obtained by size exclusion chromatography it was concluded that the time of incubation with the enzyme needed to completely degrade amylopectin molecules is short in comparison to the time needed to hydrolyse all available glycosidic bonds.

Key words: starch, pullulanase, enzymatic hydrolysis

INTRODUCTION

Unbranched glucans may be produced in several ways, that include: separation of amylose leaching from starch granules during gelatinisation [1,7], centrifugation of retrograded amylose [1,2,4], removal of amylopectin branches by enzymes [16], removal of amylopectin from starch solution [7,10,11,19], preparative ultracentrifugation [12], size exclusion chromatography [5,8], field flow fractionation, electrophoresis and some

other laboratory techniques [23]. Of those methods only the first three could be used on industrial scale [21], and the enzymatic one seems to be the most promising.

The enzymes that remove branches - isoamylase and pullulanase - catalyse the disruption of alpha-1,6 bonds which form branches in amylopectin. Isoamylase, contrary to pullulanase, does not remove side-chains containing less than 3 glucose units, so it could not be used for complete debranching of amylopectin and glycogen.

Starch preparations used in industry have traces of alpha-amylase activity that could cause partial degradation of unbranched glucans, the extent of this process must be controlled to prevent a decrease in molecular weight of amylose.

The optimal conditions for enzymatic action are provided after complete disruption of starch granules. Solubilisation procedure is then an important step of starch preparation.

The aim of the study was to compare the action of enzymatic preparation PULLUZYME 750 L depending on starch source and method of its solubilisation.

MATERIALS AND METHODS

Commercial starch samples of various botanical origin were used as a material: potato (Superior), wheat (Kroener) and high-amylose corn (Sigma). Starch was dissolved under different conditions:

1. In an autoclave - 1.5% starch suspension in water was pre-gelatinised and dissolved under 1 atm., at 121°C for 1 hour. After cooling the solution was buffered to pH 5.0 in 0.06 M acetic buffer and diluted to give 1.33% (w/v) concentration.
2. Starch was solubilised in 1.45 M NaOH and the obtained approximately 1.5% solution was buffered to pH 5.0 in 0.06 M acetic buffer according to instruction of ABM Chemicals and diluted to give 1.33% (w/v) concentration [17].
3. 0.4 g of starch was soaked with water and dissolved in 3 cm^3 of dimethylsulfoxide (DMSO) at 40°C, the obtained clear solution was diluted with water and buffered to pH 5.0 (as above). The final concentration was 1.33%w/v.
4. The influence of starch preparation method on its enzymatic hydrolysis was checked by using PULLUZYME 750 L diluted 1:100 with acetic buffer to give pH 5.0. The substrate was mixed 3:1 with the enzyme and incubated at 30°C for 15 and 45 minutes, then the content of reductive sugars in the sample was measured with 3,5-dinitrosalicylic acid (DNS) according to Wildner and Wildner [22].

The dynamics of enzymatic reaction was measured after solubilisation of starch samples in NaOH, neutralisation (HCl) and buffering to pH 5.0 with acetic buffer. The final starch concentration in substrate was 1.33% and acetic buffer 0.08M. PULLUZYME 750 L was used in 2% concentration. The substrate was heated to 30°C, mixed 3: 1 with enzyme (60 cm^3+20 cm^3) and incubated at 30°C for 90 min. The samples of 1 cm^3 were taken at specified time intervals and the enzymatic reaction was interrupted by adding 3 cm^3 of NaOH to each of them. Total reductive sugars were measured by DNS method and expressed as mg of maltose.

To describe molecular changes in potato starch 1.37% solution of this starch in 0.0625 M acetic buffer (pH 5.0) was used associated with 2.9% of PULLUZYME 750 L preparation. After 30 min of pre-heating to 30°C the substrate was mixed 5: 1 with the enzyme (30 cm^3 + 6 cm^3) and incubated at 30C removing 5 cm^3 samples after 5, 20 and 45 min to a test tube containing 1 cm^3 of 1 M NaOH to stop the reaction. In a control the enzyme was exchanged with distilled water. The final starch concentration was 0.96%. Prior to size exclusion chromatography, reductive sugars were measured with DNS and amylose content was checked with Morrison and Laignelet method [14]. The column containing Sephadex S-1000 gel was 400 mm long and 16 mm in diameter. The chromatography was performed at ambient temperature using 0.005 M Na_2CO_3 eluent at flow rate 5 cm^3/h. On-line refractometer was used as detector.

Each analysis was done in duplicate. One factor anova was perfromed with a programme Stat Skierniewice 1988

RESULTS AND DISCUSSION

In the previous work [9] of two commercial pullulanase preparations: PULLUZYME 750 L and PROMOZYME 400 L, the first was selected to further studies because of its higher (three times) activity and better pullulanase to alpha-amylase activity ratio. To find the optimal conditions of its action on starch preparation, potato, wheat and high-amylose corn starch samples were solubilised by different physical and chemical methods. The results were collected in Table 1.

Table 1. The impact of solubilisation method on starch hydrolysis under pullulanase treatment

Starch source	Solubilisation method	Degree of Hydrolysis [%]	
		after 15'	after 45'
Potato	autoclave	9.6 [c]	12.4 [c]
	NaOH	9.2 [b]	12.1 [b]
	DMSO	9.1 [a]	11.3 [a]
Wheat	autoclave	*	*
	NaOH	7.4 [a]	10.3 [a]
	DMSO	8.1 [b]	11.5 [b]
Corn	autoclave	*	*
	NaOH	5.9 [b]	7.8 [b]
	DMSO	3.1 [a]	4.0 [a]

The values obtained for selected starch and time of hydrolysis marked with different letters differ significantly ($p=0.05$)

Different solubilisation methods had the lowest impact on potato starch solubilisation, and highly changed wheat and corn starch hydrolysis. In case of wheat starch a little more pronounced effect of pullulanase action was found after its solubilisation in DMSO, whereas high amylose corn starch was almost two times more hydrolysed after use of NaOH. Regardless of substrate preparation, potato starch was most susceptible to PULLUZYME 750 L action. The probable reason of faster action of pullulanase on potato starch is the lower level of impurities in its granules [20].

The trials to liquefy cereal starch suspensions in an autoclave did not give good results, because after cooling the pastes were opalescent and heterogeneous. In case of high amylose corn starch samples the retrograded amylose precipitated. Cereal starch granules contain high levels of non-starch impurities such as lipids (wheat below 0.75%, corn about 1%), proteins (0.4% - wheat; 0.75% - corn) and pentosans [6,13]. Above starch gelatinisation temperature lipids form complexes with amylose, which similarly to pentosans, precipitate during cooling which causes opalescence [3] and can inhibit action of enzymes. To remove such dregs during production of starch syrup it is necessary to use additional enzymatic preparation: pentosanase which splits arabinoxylans and lysophospholypase that destroys amylose-lipid complexes [15,18].

Because NaOH proved to be very good solvent for two of three investigated starches and wheat starch was only slightly better solubilised in DMSO, the alkaline treatment was chosen for starch dissolving in further experiments.

The dynamics of hydrolysis in 1% starch solutions was monitored. The results are shown and sketched on Figure 1. They prove that the rate of starch hydrolysis changes due to its botanical source and imply that there is a theoretical maximum degree of starch decomposition (Fig. 1). As expressed in miligrams of maltose produced from each 100 mg of starch it is close to 24 for potato, 22 for wheat and 14 for high amylose corn starch. It is very likely that this value depends mostly on the number of branches in the starch sample and much less on the trace alpha-amylase activity present in PULLUZYME 750 L preparation [9]. It is supported by a fact that during potato starch hydrolysis the amount of amylose regularly increases (Table 2) which is accompanied by a loss of amylopectin. This is also reflected by SEC analysis, which shows excessive reduction of branched glucans with high molecular mass (Fig. 2). This is however hard to tell what is the extent of degradation caused by alpha-amylase activity.

Figure 1. Hydrolysis of starch dissolved in NaOH by pullulanaze preparation PULLUZYME 750L

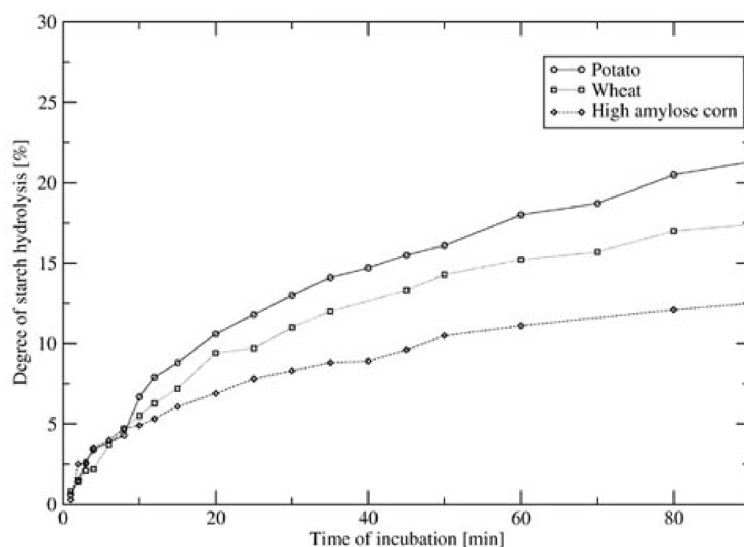

Figure 2. Size exclusion chromatography (Sephacryl S-1000 column) of glucans obtained from starch solution by hydrolysis with pullulanase

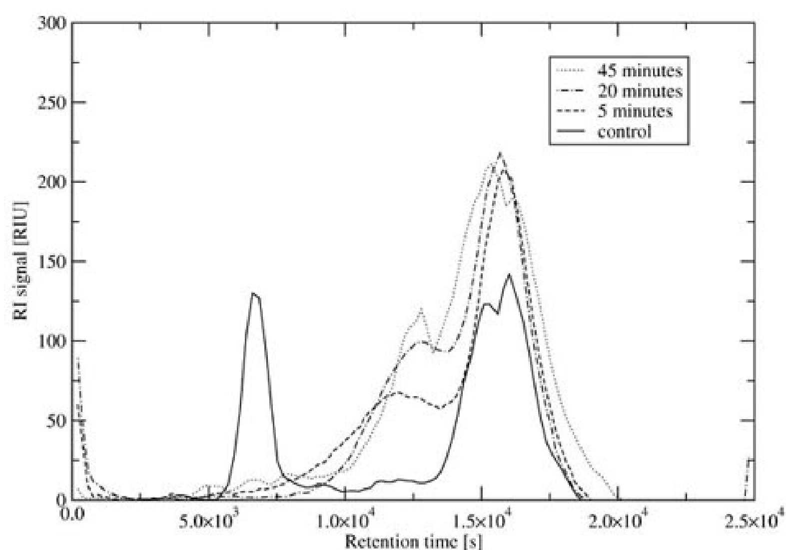

Table 2. Characteristics of potato starch after pullulanase treatment

Time of hydrolysis [min]	Degree of starch hydrolysis[%]	Amylose content [%]
0	0.6 [a]	29.5 [a]
5	3.7 [b]	32.9 [b]
20	10.6 [c]	35.4 [c]
45	16.6 [d]	36.9 [d]

The values in each column marked with different letters differ significantly (p=0.05)

Due to the highest rate of hydrolysis and its good efficiency found for potato starch, this sample was chosen for chromatography studies to characterise the length of glucans after debranching. SEC profile of untreated starch sample displays two main maxima referring to large molecules of amylopectin and much smaller amylose as well as inorganic particles (NaOH, acetic buffer). After 5 minutes of enzymatic action the maximum corresponding to large glucans completely vanishes and the broad peak appears due to a presence of products of hydrolysis of amylopectin. With further starch degradation it moves towards lower molecular weights. To some extent, it is the result of residual alpha-amylase activity in enzymatic preparation.

In order to obtain possibly longest non-branched starch chains the hydrolysis should be limited by using lower concentrations of enzyme or short incubation times.

CONCLUSIONS

5. Susceptibility of starch from different botanical sources on action of pullulanase preparation depends on the method used for starch solubilisation.
6. Potato starch, irrespective of solubilisation method, proved to be most susceptible to preparation PULLUZYME 750 L.
7. The use of autoclave for starch solubilisation is not recommended for cereal starch.
8. The alcaline treatment is recommended for starch solubilisation as the simplest and universal method.
9. During 45 min of pullulanase action the continuous decrease of molecular weight of glucans was observed, although the largest amylopectin molecules were totally destroyed after 5 minutes.

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Maciej Kujawski, Rafał Ziobro, Halina Gambuś
Faculty of Food Technology
Cracow Agricultural University, Cracow, Poland
29-Listopada 46, 31-425 Cracow, Poland
tel./fax: (+48 12) 633 62 45
e-mail: rrziobro@cyf-kr.edu.pl

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