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# RAW STARCH DEGRADATION BY PULLULANASE

Maciej Kujawski, Rafał Ziobro, Halina Gambuś

**Abstract:** Activity of two commercial pullulanase preparations has been compared with biochemical grade enzyme from Sigma. Of them PULLUZYME 750 was chosen as a reagent for hydrolysis of raw starch granules from different botanical species. The obtained results suggest that high amylose corn starch and native potato starch are more resistant to pullulanase action than wheat starch which under the action of this enzyme becomes slightly degraded.

**Key words:** starch, enzymatic hydrolysis, pullulanase

## INTRODUCTION

Starch is a biopolymer consisting of glucose residues connected into large macromolecules: linear amylose, where they are bound almost exclusively through alpha-1,4-glycosidic bonds and amylopectin, where alpha- 1,6-glycosidic form about 5% connections, that causes significant branching of this polymer [Thompson 2000].

Industrial use of starch often requires their depolymerisation, which could be done chemically or enzymatically. Traditional chemical hydrolysis has been prevailed by enzymatic treatment, because of the following advantages:

– better controlling of physical and chemical properties of the products,

– fewer side reactions, causing unwanted changes like browning [Nigam and Singh 1995, Crab and Mitchinson 1997]

Enzymes used in industry can hydrolyse alpha-1,4-glycosidic bonds (alpha-, beta- and glucoamylases of different origin) or amylopectin branches (glucoamylase, isoamylase and pullulanase). The majority of hydrolysing enzymes have only limited capability to attact native starch granules [Nowotny 1938], however the high energy input required for starch gelatinisation stimulates the search for amylases attacking raw starch granules [Nigam and Singh 1995].

The studies on hydrolysis of alpha-1,4-glycosidic bonds in native starch has been focused on alpha-amylase and glucoamylase. The similar experiments with alpha-1,6-glycosidases has been done only for isoamylase [Kimura and Robyt 1996].

Due to the wide use of pullulanase in industry [Nigam and Singh 1995], the study focused on the possible use of commercially available pullulanase preparations to degrade unprocessed starch granules.

## MATERIAL AND METHODS

Two commercial pullulanase preparations were used: PULLUZYME 750 L (UBICHEM Ltd., England) and PROMOZYME 400 L (NOVO NORDISK A/S, Denmark) in the form of liquid concentrate. Biochemical grade lyophilised enzyme was obtained from Sigma. Enzymatic activity was checked with pullulan standard and chemically pure potato amylose from Sigma. The substrates were: potato starch – Superior (Poland), wheat starch – Kroener (Germany) and high amylose corn starch from Sigma.

Amylose content in starch samples was measured according to Morrison and Laignelet [1983]. Standard deviation of the measurement was 0.44.

Pullulanase and alpha-amylase activities were calculated according to the instructions of Pulluzyme-... [1977]. The suspension of enzyme was incubated in 1% pullulan or amylose solution adjusted to pH 5.0 by adding 0.05 M acetic buffer at 30°C, for 15 minutes. The resulting reductive sugars were quantified using 3,5-dinitrosalicylic acid, according to Wildner and Wildner [1959] and expressed as the amount of maltose. The amount of reducing sugars equ to one miligram of maltose produced in 1 minute under described conditions was taken as activity unit.

Treatment of starch with pullulanase was performed in 4% w/v starch dispersion in 2% w/v enzyme solution, buffered to pH 5.0. The incubation at 40°C was conducted for 16 hours with gentle stirring (160 rpm). Then starch was rapidly cooled and centrifuged, and the obtained supernatant was filtered through a paper filter and total soluble carbohydrates were measured using anthrone method [Koehler 1952]. Precipitated starch was thoroughly washed in centrifugal tubes with distilled water (3 times), ethanol (2 times), absolute ethanol (2 times) and acetone. Then it was dried in a dryer (2h, 37°C) and weighed. Control samples were prepared by exchanging enzyme with acetic buffer.

## RESULTS AND DISCUSSION

The basic characteristic of starch samples used in study is shown in Table 1. Commercial wheat and potato starches did not significantly differ in amylose content, while corn starch was much more abundant in this polymer.

Table 1. The characteristics of native starch samples
Tabela 1. Charakterystyka próbek skrobi naturalnej

| Starch sample<br>Próbka skrobi | Dry mass, %<br>Sucha masa, % | Amylose, %<br>Amyloza, % | Amylopectin, %<br>Amylopektyna, % |
|---|---|---|---|
| Potato<br>Ziemniaczana | 86.18 | 19.4 | 80.6 |
| Wheat<br>Pszenna | 88.84 | 16.7 | 83.3 |
| High amylose Corn<br>Wysokoamylozowa kukurydziana | 88.41 | 53.5 | 46.4 |

Table 2 contains the results of enzymatic activity measurements. All preparations contained traces of alpha-amylase. Biochemical grade pullulanase from Sigma displayed much higher activity than commercial ones. The ratio of pullulanase to alpha-amylase was also much more favourable in this case and equaled 170:1. Commercial preparations were less active, higher activity and pullulanase to alpha-amylase ratio more appropriate in long chain preparation were found for PULLUZYME 750 L. Thus it was used in further experiments because biochemically pure enzymes are too expensive to be used on a larger scale.

Table 2. The characteristics of pullulanase (E.C.3.2.1.41) preparations
Tabela 2. Charakterystyka preparatów pullulanazy (E.C.3.2.1.41)

| Name<br>Nazwa | Source of enzyme<br>Źródło enzymu | Pullulanase activity<br>Aktywność pullulanazy | | Alpha-amylase activity<br>Aktywność alfa-amylazy | | Pullulanase to alpha-amylase activity ratio<br>Stosunek aktywności pullulanazy do alfa-amylazy |
|---|---|---|---|---|---|---|
| | | 1 $cm^3$ | 1 mg | 1 $cm^3$ | 1 mg | |
| Pulluzyme 750L | *Klebsiella aerogens* | 327.6 | 0.2792 | 7.940 | 0.0068 | 41:1 |
| Promozyme 400L | *Bacillus acidopullulyticus* | 104.7 | 0.0842 | 4.614 | 0.0037 | 22:1 |
| Pullulanase Sigma | *Klebsiella pneumoniae* | – | 3.536 | – | 0.0207 | 171:1 |

The trials to employ pullulanase for raw starch degradation did not give practically significant results. In case of all starch samples, some loss of amylose fraction was observed (Table 3) – in case of potato starch in the range of method's error – that can be due to penetration of alpha-amylase into granules. At the same time, in potato and corn starch supernatants some (under 5%) soluble sugars were found which proves that occurs only limited hydrolysis of alpha-1,6-glycosidic bonds. It may be because of the inability of the enzyme to penetrate the channels (because of their size) or bond to the outer surface of starch granules [Fannon et al. 1992, Kimura and Robyt 1996, Fortuna et al. 2000].

Table 3. Raw starch degradation under pullulanase treatment
Tabela 3. Degradacja skrobi surowej w wyniku działnia pullulanazy

| Starch sample<br>Próbka skrobi | Mass, mg<br>Masa, mg | | Soluble sugars, mg<br>Cukry rozpuszczalne, mg | | Amylose, %<br>Amyloza, % |
|---|---|---|---|---|---|
| Potato – Ziemniaczana | 1 639 | | 25.9 | | 21.0 |
| – before treatment<br>– przed działaniem | 1 682 | – 43 | 1.8 | +24.1 | 21.4 |
| Wheat – Pszenna | 1 518 | | 133.6 | | 17.6 |
| – before treatment<br>– przed działaniem | 1 645 | – 43 | 10.2 | +123,4 | 18.1 |
| Corn – Kukurydziana | 1 596 | | 43.9 | | 52,8 |
| – before treatment<br>– przed działaniem | 1 637 | – 41 | 0.51 | +38,8 | 54.4 |

The results displayed in table 3 show that different native starches are affected by pullulanase in various extent and are consistent with the data regarding raw starch susceptibility to alpha-amylase and glucoamylase, which point out potato and high-amylose starches as especially resistant to enzymes [Sugimoto et al. 1980, Kimura and Robyt 1995, Sawicka-Żukowska et al. 1999]

The loss of starch mass due to hydrolysis in case of wheat and corn starch did not differ from the numbers for total carbohydrates in supernatant. In corn and potato starch it was three times lower in comparison to wheat starch, while the decrease in amylose was comparable for wheat and potato starch. It seems then, that relatively large decrease of starch mass (8%) and release of water soluble sugars observed for wheat was significantly affected by degradation of amylopectin by pullulanase.

This is in accordance with the report of Sawicka-Żukowska et al. [1999] who observed that among different starch sources, the most susceptible to amylolytic enzymes are wheat and corn. Basing on the results of Sreenath [1992] one can then expect, that the surface of wheat starch granules is modified by alpha-amylase action. Such changes can lead to development of larger surface structures easily attacked by other enzymes such as pullulanase.

## CONCLUSIONS

1. Of the used pullulanase preparations, the highest activity was found for biochemically pure enzyme from Sigma.
2. All the examined preparations had trace activity of alpha-amylase.
3. The commercial preparation chosen for further studies – PULLUZYME 750 L exhibited higher activity and pullulanase to alpha-amylase ratio more appropriate for obtaining longer amylose chains, than the other one.
4. Raw wheat starch was most susceptible to pullulanase action.

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## DEGRADACJA SKROBI SUROWEJ PRZEZ PULLULANAZĘ

**Streszczenie:** Porównano aktywność dwóch handlowych preparatów enzymatycznych z biochemicznie czystym enzymem z firmy Sigma. Wybrany preparat PULLUZYME 750 został użyty do hydrolizy ziarenek surowej skrobi różnego pochodzenia botanicznego. Uzyskane wyniki wskazują, że wysokoamylozowa skrobia kukurydziana i skrobia ziemniaczana są bardziej oporne na działanie pullulanazy niż skrobia pszenna, która pod wpływem tego enzymu ulega nieznacznej degradacji.

**Słowa kluczowe:** skrobia, hydroliza enzymatyczna, pullulanaza

*M. Kujawski, R. Ziobro, H. Gambuś, Wydział Technologii Żywności, Akademia Rolnicza w Krakowie, al. 29 Listopada 46, 31-425 Kraków*