Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series FOOD SCIENCE AND TECHNOLOGY

SŁOMIŃSKA L., KLISOWSKA M., GRZEŚKOWIAK A. 2003. DEGRADATION OF STARCH GRANULES BY AMYLASES
Electronic Journal of Polish Agricultural Universities, Food Science and Technology, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

DEGRADATION OF STARCH GRANULES BY AMYLASES

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ABSTRACT

Production of enzymatic starch hydrolyzates is made by two-steps action of amylolytic enzymes on gelatinized starch granules – at the beginning by liquefying enzymes and next by saccharifying enzymes. Differences of optimal parameters of the enzyme action require the change of reaction conditions: temperature and pH. The aim of the study was simultaneous action of some enzymes, α -amylase, glucoamylase and pullulanase, on hydrolysis of raw starch. The various combinations of amylolytic enzymes in different reaction parameter were used during hydrolysis of potato, wheat and corn starch in production of glucose hydrolyzates. Application of direct enzymatic action on non-gelatinised starch create high degree of saccharification (DE) – only some dextrose equivalent less than obtained during traditional two-steps process of starch hydrolysis. The highest susceptibility to degradation by enzyme action indicates granules of corn starch, the lowest potato starch.

Key words: raw starch, enzyme, dextrose equivalent, starch hydrolyzates.

INTRODUCTION

Technology of starch hydrolyzates includes two steps of hydrolysis : liquefaction in which insoluble starch is converted into soluble polymer fragments and saccharification step in which it is carried further break of these fragments into the desired starch sugars. Liquefaction is done by termostable alpha-amylase initially at 105°C followed by incubation at 95°C (pH 5.5 - 6.5) and takes 2-3 h. Saccharification is made at 60°C (pH 4.5 – 5.0)

by glucoamylase and pullulanase action and takes 48 - 72 h. [3,4,5,7,10,17,19,20,21,22]. Different optimum conditions of liquefying and saccharifying enzyme action required changes of pH and temperature.

The aim of this study is to relate the degree of starch conversion by synergistic action of liquefying and saccharifying enzymes on starch granules – the alternative way of starch hydrolyzate production to traditional technology.

MATERIALS AND METHODS

Starch

Soluble starch (Merck), potato starch (Factory of Potato Industry – Poland), corn starch (Amylum Belgia) and wheat starch (Factory of Potato Industry – Poland) were used.

Enzyme

Termamyl 120 type LS (Novo Nordisk, Denmark) - a mixture of outstanding heat-stable alpha-amylases (EC 3.21.1) produced by selected strains of *Bacillus licheniformis.* The enzyme activity was 120 KNU/g (KNU = Kilo Novo Units alpha-amylase – the amount of enzyme which breaks down 5.26 g of starch per hour at Novo's standard method for determination of alpha amylase).

Dextrozyme E (Novo Nordisk, Denmark) – a balanced mixture of glucoamylase and pullulanase obtained from genetically modified strains of *Aspergillus niger* and *Bacillus deramificans*.

AMG 300L (Novo Nordisk, Denmark) an amyloglucosidase (ES 3.2.1.3.) produced by genetically modified strain of an *Aspergillus* microorganism.

The enzyme activity was 300 AGU/ml. (AGU = the amount of enzyme which hydrolysis 1µmol maltose per minute under following conditions: substrate - maltose, temperature 25°C, pH 4.3, reaction time 30 min).

Promozyme 200L (Novo Nordisk, Denmark) – a heat stable debranching enzyme obtained from a selected strain of *Bacillus acidopullulyticus.*

The enzyme activity was 200 PUN/g (PNU = the amount of enzyme which hydrolyses pullulan and liberats reducing carbohydrate with a reducing power equivalent to 1 micro-mol glucose per minute under standard conditions).

Termadex (Genecor, US) - a mixture liquefying and saccharifying enzymes obtained from the genus *Humicola*

Procedure

10 % (w/w) soluble (solution) or raw (suspension) starches were treated by mixture of various enzymatic preparations which were used with different dosages at temperature 60°C and pH 5.0 for 72 h. Comparative hydrolysis of starch was carried out in two steps method: liquefaction at 95°C and pH 6.5 and saccharification at 60°C and pH 4.5.

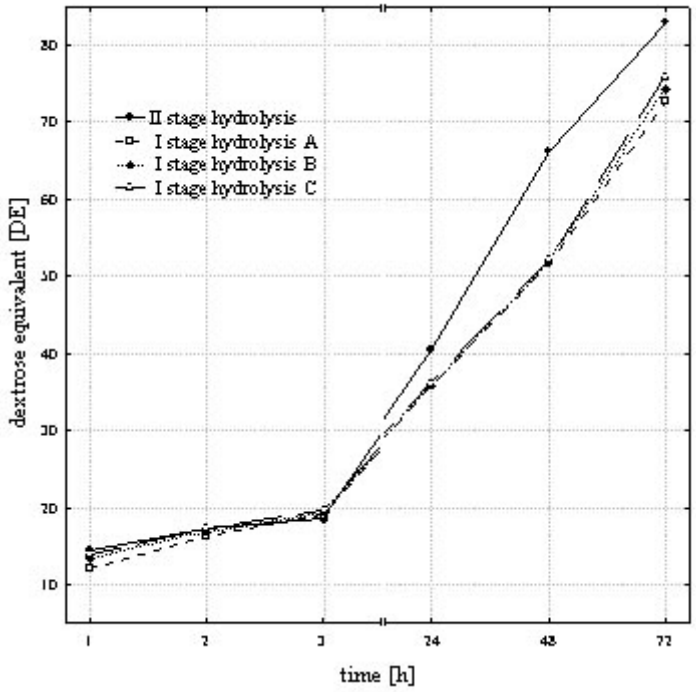
Dextrose equivalent was estimated by modified Schoorla-Rogenbogen method [16]. Reducing sugars in the hydrolyzates were determined by 3,5-dinitrosalicylic acid method and represented in terms of glucose [15].

RESULTS AND DISCUSSION

Enzyme dosage

Comparison of hydrolysis of soluble starch carried out by two-stage degradation with application of liquefying enzyme (Termamyl 120l – 0.1%) at temperature 95°C and pH 4.5 and saccharifying enzyme (Dextrozyme E – 0.1%) at temperature 60°C and pH 5.0 and hydrolysis carried out by simultaneous action of these enzymes used in the same dosages but different reaction conditions: at temperature 60°C and pH 5.0 was made. It indicates that the difference of dextrose equivalent obtained hydrolyzates after 72h of reaction was 10 DE. The increase of enzyme dosage (Dextrozyme E) by 50% during simultaneous action of used enzymes decreases amount of dextrose equivalent till 8.5 DE but higher increase of enzyme dosage (two-times) decreases it till 6.9DE (Fig. 1)

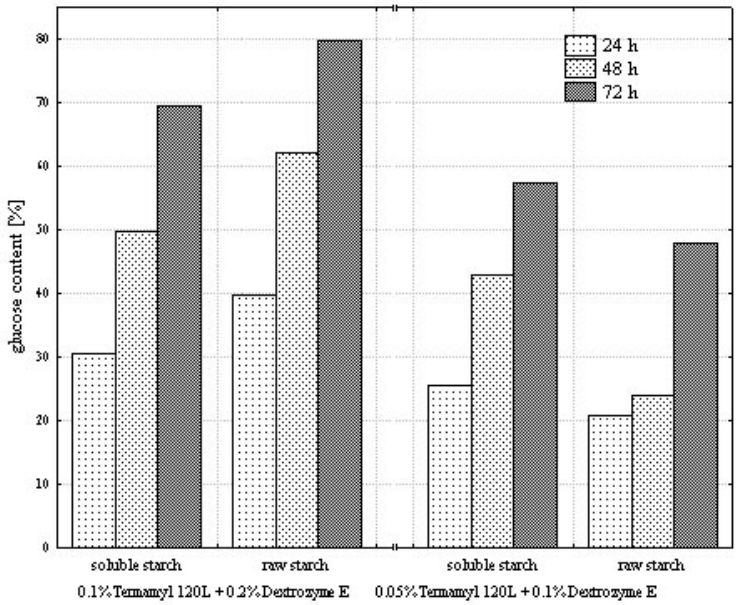
Fig. 1. Hydrolysis of soluble starch by simultaneous and separately action of amylolitic enzymes.
Process conditions: single-stage hydrolysis: 60°C, pH 5.0, enzyme dosages: A: 0.1% Termamyl 120L + 0.1% Dextrozyme E; B: 0.1% Termamyl 120L + 0.15% Dextrozyme E; C: 0.1% Termamyl 120L + 0.2% Dextrozyme E; two-stage hydrolysis: liquefaction - Termamyl 120L (0.1%), 3h, 95°C, pH 6.5; saccharification - Dextrozyme E (0.1%) 72 h, 60°C, pH 4.5



Kind of starch

Amount of glucose which is obtained after simultaneous action of Termamyl 120L and Dextrozyme E on soluble and raw potato starch indicates that when there is the same rate (1:2) of these enzymatic preparations but various dosages are applied, the enzymatic susceptibility of starches is different (Fig. 2). In the case of application of smaller enzyme dosages (Termamyl - 0.05% and Dextrozyme E – 0.1%) the amount of glucose after 72 h hydrolysis of raw starch is by 6.6% lower than after hydrolysis of soluble starch. The use of two-times higher dosages of the preparations influences on higher enzymatic susceptibility of raw starch than soluble starch. The obtained from raw starch hydrolyzate has by 10.2% higher glucose than hydrolyzate obtained from soluble starch.

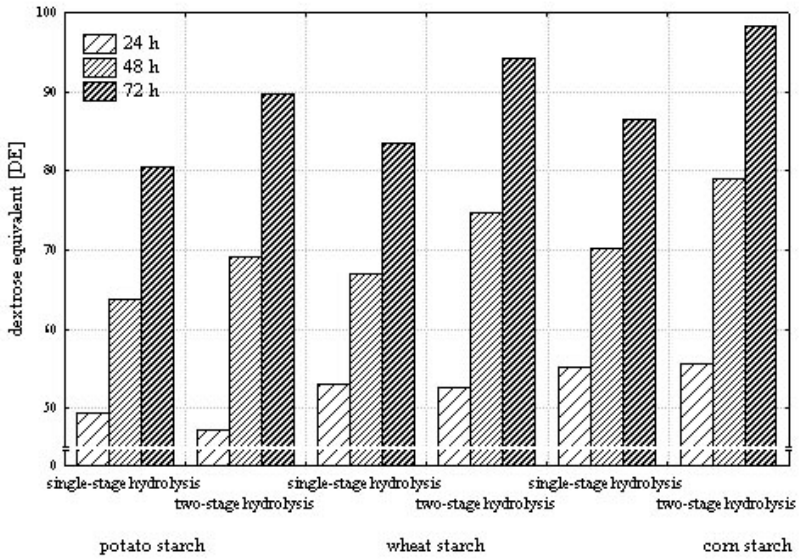
Fig. 2. Comparison of glucose content obtained by simultaneous action of Termamyl 120L and Dextrozyme E on soluble and raw potato starch. Process conditions: substrate concentration - 10 % starch, 60°C, pH 5.5, enzyme dosages: 0.05% Termamyl 120L + 0.1% Dextrozyme E, 0.1% Termamyl 120L + 0.2% Dextrozyme E



The comparison of simultaneous action of Termamyl 120L (0.1%) and Dextrozyme (0.1%) on raw potato, corn and wheat starches makes different enzymatic susceptibility of starches. Corn starch was the most susceptible and potato starch the least.

Two-stage hydrolysis of starches creates the similar sequence of their susceptibility but DE is higher. After 72 h enzyme reaction the differences of dextrose equivalent for potato, wheat and corn hydrolyzates amounts 9.4, 10.7 and 12.0, DE, respectively. The disagreements of DE appear just after 48 h of reaction (Fig. 3).

Fig. 3. Single- and two-stage hydrolysis of raw starch.
Process conditions: Single-stage hydrolysis: 60°C, pH 5.0, enzyme dosages: 0.1% Termamyl 120L + 0.1% Dextrozyme E; two-stage hydrolysis: liquefaction - Termamyl 120L (0.1%), 3h, 95°C, pH 6.5; saccharification - Dextrozyme E (0.1%), 72h, 60°C, pH 4.5

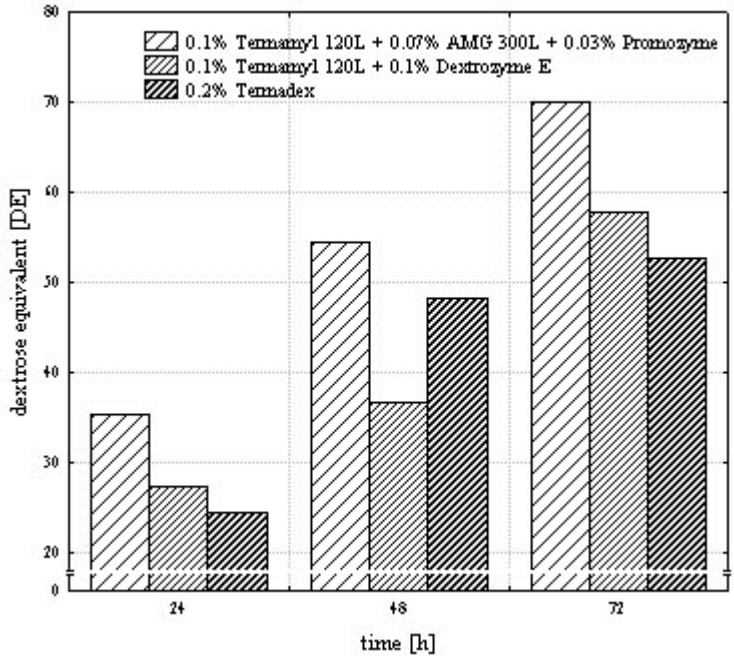


Kind of enzymes

Figure 4 illustrates the degree of saccharification reaction of raw corn starch by application of the following enzymatic preparations: I set: Termamyl 120l+ AMG 300L + Promozyme 200L, II set: Termamyl 120L + Dextrozyme E, III set Termadex:

The complete identical dosage of enzyme set used during hydrolysis of raw corn starch leads to the obtainment of the highest DE after 72h of hydrolysis at 60°C with the use of I set of enzymes. II and III sets give hydrolyzates with lower by 17.2 and 24.9% respectively.

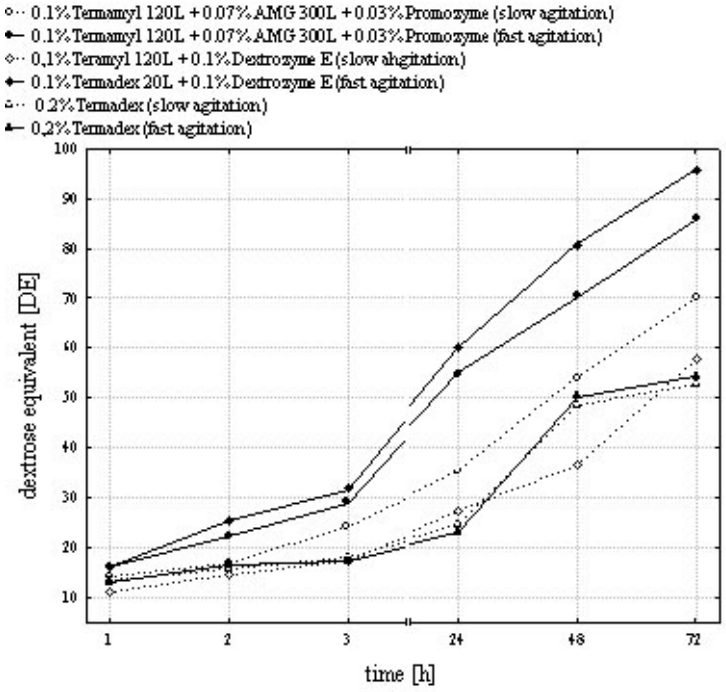
Fig. 4. Influence of enzymatic composition on depolimerization of corn starch.
Process conditions: substrate concentration - 10% starch, 60°C, pH 5.0, enzyme dosages: I: 0.1% Termamyl 120L + 0.07% AMG 300L + 0.03% Promozyme; II: 0.1% Termamyl 120L + 0.1% Dextrozyme E; III: 0.2% Termadex



Speed of mixing

The introduction of higher agitation rate during hydrolysis of raw potato starch influences in different extent on DE value of hydrolyzates. In the case of I set application the dextrose equivalent after 72h of reaction increases by 15.7, II set by 38.1 and III set by 6 DE (Fig. 5).

Fig. 5. Influence of agitation speed on potato starch hydrolysis. Process conditions: substrate concentration - 10% starch, 60°C, pH 5.0, enzyme dosages: I: 0.1% Termamyl 120L + 0.07% AMG 300L + 0.03% Promozyme; II: 0.1% Termamyl 120L + 0.1% Dextrozyme E; III: 0.2% Termadex



Action of amylolitic enzymes on raw starch

The evaluation of influence of enzymes on raw starch was the subject of interest of some authors.

Arasaratman and others [1] hydrolyzed starch in corn flour by the synergistic action of alpha-amylase (Termamyl 60L) and glucosidase (Spiritamylase) at 70°C. When 16% suspension of corn flour was hydrolyzed glucose yield was 76.0% but when 40% suspension only 50.2%.

Arasaratman and Balasubramanian [2] treated waxy corn starch by mixture of alpha-amylase and glucoamylase at 70°C. Hydrolysis efficiency was 99.2%. The same level of hydrolysis was also obtained by enzymatic treatment of 18.5% corn flour suspension.

Karakatrnis et. al. [11] used the combination of alpha –amylase and glucoamylase action for hydrolysis of soluble starch and crude corn and rice starches. They hydrolyzed 5% starch suspension at different temperatures (30, 40, 50, 60°C) in an impeller agitated bioreactor (150 rpm). After 5h of enzymatic action at 40°C the conversion of corn starch to glucose was almost 100%, rice starch 79.27% and soluble starch only 36.92%.

Previous research confirm the result obtained in the present study that cereal-starch granules are susceptible, whereas potato-starch granules are resistant to hydrolysis.

Karakatsanis and Liakopoulus-Kyriakidis [12] used simultaneous action of alpha-amylase and glucoamylase during hydrolysis of various starches. After 24 h reaction at 40°C glucose production was following: 96.0% - corn starch, 93.2% - rice starch, 85.3% - barley starch, 59.8% - wheat starch, 48.3% - potato starch, 685.6% - soluble starch.

Differences of susceptibility between cereal and potato starches confirmed also study carried out by Fuwa [8,9]. Leach and Schoch [14] inform that higher susceptibility on alpha-amylase action has raw corn starch than high amylose corn starch.

Kimura and Robyt [13] studied also influence of glucoamylase (*Rhizpus niceus*) on starch granules from different botanical sources (waxymaize, barley, tapioca, potato, corn). Hydrolysis was carried at 37°C for 32h for three concentrations of enzyme. Waxymaize starch was the most susceptible being converted into 98% D-

glucose. Starches from barley, maize and tapioca were converted into 10-15, 60-and 75-80% D-glucose. The potato starch was the least susceptible (13-21% D-glucose).

Sawicka-Żukowska et al. [18] present result of alpha-amylase (Amylopol SC-15, Termamyl 120L) and glucoamylase (Glukopol P-15) action on soluble starch and raw potato, corn and wheat starches. Degree of hydrolysis was the highest for wheat starch and amounted in the case of alpha-amylase action 13-18% and in the case of glucoamylase action 32-35%. The lowest degree of hydrolysis indicated potato starch (1-2% -alpha amylase action, 1-4% -glucoamylase action).

Dettori-Campus and et al. [6] hydrolysed corn and wheat starch granules by amylase from *Bacillus staetherophilus*. Digestion performed at 40°C gave low activity on potato starch granules (25% conversion after 100h) and high on barley, corn and rice starches (63-80% conversion after 50 h). When temperature was increased to 60°C hydrolysis of potato starch granules was more effective and 45% conversion to glucose was achieved in 12 h.

CONLUSIONS

1. Simultaneous action of liquefying and saccharifying enzymes on raw starch gives comparable degree of hydrolysis to separately action of enzymes on gelatinized starch.
2. Application of Termamyl 120L (0.1%) and Dextrozyme E (0.1%) action during hydrolysis of raw potato starch gives hydrolyzate with lower only by 9 DE than hydrolyzate obtained by traditional two-steps hydrolysis of gelatinized starch.
3. Potato starch granules is more resistant to amylase digestion than corn and wheat starches.
4. The increase of speed of agitation influences on enhancement of degree of starch hydrolysis.
5. Application of similar parameters of liquefying and saccharifying enzyme action (pH, temperature) influence on the decrease of ion amount, which are introduced during pH changes and the decrease of energy consumption connected with solubilization and gelatinization starch.

ACKNOWLEDGMENTS

This study has been carried out with financial support from the State Committee of Scientific Research (grant PBŻ-KBN 021/P06/13/2001)

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