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THE EFFECT OF PROCESS CONDITIONS OF ENZYMATIC HYDROLYSIS ON THE PROPERTIES OF WHEAT STARCH HYDROLYZATES

Abstract

In the study, glucose hydrolyzates were obtained by acting on wheat starch, which was liquefied with the aid of bacterial α -amylase, with Spezyme GA 300W Y553, an enzymatic preparation composed of glucoamylase and lysophospholipase, as well as with Amyloglucosidase AMG 300L, a glucoamylase preparation. Parallel to saccharifying enzymes, Gammazym CX 4000L, Shearzyme 500L and proteinase Neutrase 0.5L, cellulolytic enzymes, were used.

The best physical and chemical properties of the obtained glucose hydrolyzates: the colour factor, the transparency factor, the filtration power, and the highest reductivity were obtained in the process of saccharification using, beside Spezyme, the xylanase preparations: Shearzyme 500L and proteinase Neutrase 0.5L.

Introduction

The technology of starch hydrolyzates production depends on the origin of starch which is connected with the shape and size of starch grains, amylose-to-amylopectin ratio and chemical composition. Differences observed in the content of protein, fat and non-starch polysaccharides, e.g. pentosanes in raw starch subjected to hydrolysis, depending on its origin, affect the quality of hydrolyzates obtained.

In our country potato starch was the basic raw material in starch industry in the past. Now, for economic reasons grain starch is used to produce hydrolyzates. Wheat and corn starch reveals different physical and chemical properties than potato starch. Particularly, the presence of a larger amount of fat – lysophospholipids (wheat starch) and fatty acids (corn starch) which form gelating complexes with amylose, and proteins and non-starch polysaccharides (arabinoxylanes) in wheat starch has a negative effect on the colour, transparency and aroma of the hydrolyzates obtained.

Wheat starch hydrolyzates are characterised by high resistance during the process of filtration. This calls for adding enzymes enhancing the hydrolysis of the above mentioned compounds to the process of grain starch hydrolysis [2, 4, 8, 9, 11].

Grain starches contain considerably more fat impurities (wheat 1.12% in this number 62% of lysolecithin, corn 0.87% with 17% lysolecithin) as compared to potato starch (0.05%) or tapioca starch (0.1%) [1, 9]. The lipid fraction of wheat starch is mainly composed of lysophospholipids, whereas that of corn starch, of free fat acids.

During heating of starch suspension at gruelling temperature or higher, fats form two types of amylose-fat complexes: amylose-lysolecithin and amylose-fatty acids (palmitin acid, lynolene acid) [4, 9]. Each percent of fat forms from 10 to 20% of starch-fat complexes, the percentage depending on the composition of fat. [2].

Amylose in this state is resistant to the action of amylytic enzymes and swells only slightly during gruelling, due to reduced water-fixing power. Lipids account also for clouding of hydrolyzates, an unpleasant taste and odour caused by oxidation of fats, and a decrease in the filtration rate of hydrolyzates [9].

A disadvantageous influence exerted by fat on the production process of corn and wheat hydrolyzates may be diminished by utilising the action of a lysophospholipase enzyme which catalyses the reaction of decomposition of amylose-fat complexes [4,5]. It decomposes lysophospholipids to fatty acids and glycerophosphocholine (with the formation of lysolecithins). In this way the ability of lysophospholipids to form emulsions is destroyed. Due to this the colour and clarity of hydrolyzates is improved, the filtration rate increases and the quantity of filtration assistance used is reduced [4].

From the point of view of technology, contamination of the starch with proteins which can produce unpleasant flavour and contribute to foaming during gruelling and stain starch hydrolyzates, is also disadvantageous.

A specially large quantity of proteins accompany grain starches, i.e. wheat starch (0.4%) and corn starch (0.35%), in comparison with potato starch (0.06%) [8, 9].

In water media proteins form difference complexes with polysaccharides. In these complexes there are ionic bonds. However, inert polysaccharides form with proteins hydrogen and hydroxyl bonds [3].

At the concentration of macromolecules exceeding 1%, in the biopolymer systems a division of the system into two liquid phases was observed: one containing only protein, while the other one only polysaccharide. This is a generally observed phenomenon, however not common to all types of starch. It refers only to grain starch and not to potato starch which irrespective of the pH value, yields a one-phase system. Thermodynamic adjustment of proteins and inert polysaccharides reduces with pH approaching the isoelectric point of proteins and with an increasing molecular weight of polysaccharide [12].

As distinguished from potato starch and corn starch, the chemical composition of wheat starch is characterised by the presence of non-starch polysaccharides – these are mainly pentosanes, i.e. the polymers of β -1,4-xylose with β -1,3-arabinose branchings.

These substances are characterised by high water absorption in the aqueous solution forming gel substances of high viscosity, and also by the ability to form complexes with proteins which cannot be removed using physical procedures only. They are a special obstacle in the process of filtration of hydrolyzates obtained by enzymatic hydrolysis [2].

Disadvantageous properties of pentosanes in the production process of enzymatic starch hydrolyzates can be reduced by applying pentozanase enzyme. It decomposes arabinoxylanes, bringing about a reduction of viscosity and an increase of the filtration rate. It also constrains clouding in the process of cooling the hydrolyzate (as a result of precipitation of xylanases at low temperature) and changes the structure of residue by transforming non-soluble pentosanes into soluble ones [2, 6].

The aim of this study was to obtain from wheat starch such glucose hydrolyzates which would have the optimum physical and chemical properties, i.e. good filtration ability, transparency, and lack of colour, the features reached due to the additional application of lysophospholipase, proteinase and cellulolytic enzymes in the process of enzyme hydrolysis. They enhance the hydrolysis of fat, proteins and arabinoxylanes.

Materials and analytical methods

The substrate used for research purposes was wheat starch containing 88.2% of dry matter, of pH = 6.4, of acidity equal to 1.3°N, alkalinity equal to 1.1°N, fat content equal to 1.0%, protein content equal to 1.1% and ash content equal to 0.2%, in relation to dry matter.

For research purposes, the following enzymatic preparations were used in the tests:

- α -amylase TERMAMYL 120L manufactured by Novo Nordisk A/S, of an activity equal to 240 KNU/g, (pH = 5.0-6.5, 80-105°C),
- glucoamylase AMG 300L manufactured by Novo Nordisk A/S, of an activity equal to 300 AG/cm³, (pH = 3.5-5.5, 25-60°C),
- SPEZYME GA 300W Y553, an enzymatic preparation manufactured by Gamma Chemie GmbH, containing glucoamylase and lysophospholipase in its composition, of an activity equal to 300 SGU/ml, (pH = 3.8-4.5, 60°C),
- xylanase-cellulase GAMMAZYM CX 4000L Y552 manufactured by Gamma Chemie GmbH, (pH = 4.0-6.0, 40-55°C),
- xylanase SHEARZYME 500L, manufactured by Novo Nordisk A/S, of an activity equal to 500 FXU/g, (pH = 4.0-5.0, 50-70°C),

- proteinase NEUTRASE 0.5L, manufactured by Novo Nordisk A/S (pH = 5.5-7.0, 25-55°C).

Analytical methods

The analysis of enzymatic wheat hydrolyzates involved carrying out the following determinations:

- the content of reductive sugars, using the Lane-Eynon method [10], after a previous elimination of proteins from hydrolyzates;
- the filtration rate of wheat hydrolyzates;

(For determination purposes, always the same volume of hydrolyzate, equal 90 cm³, was drawn and the concentration of each of the samples brought to the value of 37°Bx. At the temperature of 60°C, the hydrolyzate was filtered through fluted filter paper of a diameter that was constant for all samples and equal to 205 mm and of the area of 33011 mm². The filtration area was equal to 31420 mm². Measurements of the filtrate volume obtained after 5, 10, 15, 20 and 25 minutes were taken.)

- the colour factor of the solution [7];

(Measurements of solution absorbency were taken at the length of the light wave equal to 400 and 720 nm and calculated in terms of 1 g of product and a layer of 1 cm in thickness. Absorbency measurements were taken using a "Spekol" spectrophotometer, after filtering, at pH equal to 5.6-5.7 and the concentration of hydrolyzates equal to 30° Bx.)

- the transparency factor of the solution [7].

(The determination was carried out by preparing wheat hydrolyzate solutions as above and using a "Spekol" spectrophotometer, absorbency measurements were taken, at the length of the light wave equal to 720 nm, in relation to distilled water.)

The process of hydrolysis

Production of glucose hydrolyzates out of wheat starch was carried out in the two following stages: liquefaction and saccharification of starch. For this purpose, the hydrolysis was carried out using the enzyme-enzyme method.

Liquefaction of a suspension of starch, of a concentration equal to 33%, was carried out at pH = 6.5, at the temperature of 95°C and the concentration of α -amylase enzyme equal to 0.15%/d.m.

During saccharification the enzymatic preparation Spezyme GA 300W Y552 of concentration 0.3%/d.m., containing glucoamylase and lysophospholipase was used. For comparison the glucoamylase preparation AMG 300L (concentration 0.3%/d.m.) was applied.

Jointly with these preparations cellulolytic enzymes (xylanase-cellulase Gammazym CX 4000L – concentration 0.06%/d.m. and xylanase Shearzyme 500L – concentration 0.069%/d.m.) and proteolytic enzyme (Neutrase 0.5L – concentration 0.013%/d.m.) were used.

Results and discussion

Results of analysis of wheat starch hydrolyzates obtained by acting on liquefied wheat starch with Spezyme GA 300W, containing glucoamylase and lysophospholipase and with cellulolytic enzymes and proteinase as compared to glucoamylase preparation Amyloglucosidase AMG 300L, are illustrated in Graphs 1 through 5.

With Spezyme (glucoamylase and lysophospholipase complex) used in the process of saccharification jointly with various cellulolytic preparations and proteinase, a much higher degree of saccharification was obtained (of the order of 99-99.7DE) than when using the same system with the application of glucoamylase AMG 300L, a saccharifying enzyme, where reductivity reached 97.9 to 98.4 DE. Lysophospholipase, due to the decomposition of amylase-lipid complexes, and cellulolytic enzymes, due to the destruction of cell walls of starch grains, enhance the access of amylolytic enzymes to starch. As a result, higher reductivity is obtained when Spezyme and assisting enzymes are used.

The dependence of saccharification degree of glucose hydrolyzates on the time of hydrolysis, and on the system of enzymes used, is shown in Graph 1.

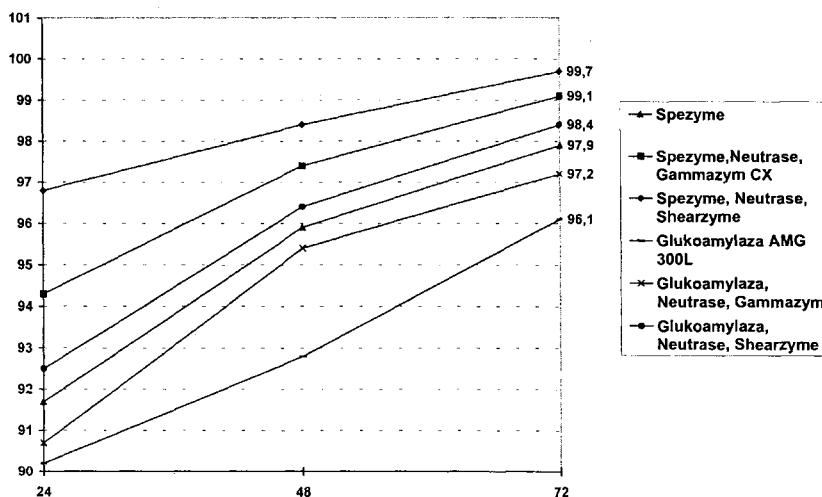


Diagram 1. Reducibility of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with on additive of various cellulolytic preparations and of a proteolytic preparation.

X: Duration of hydrolysis [h], Y: Degree of saccharification [DE]

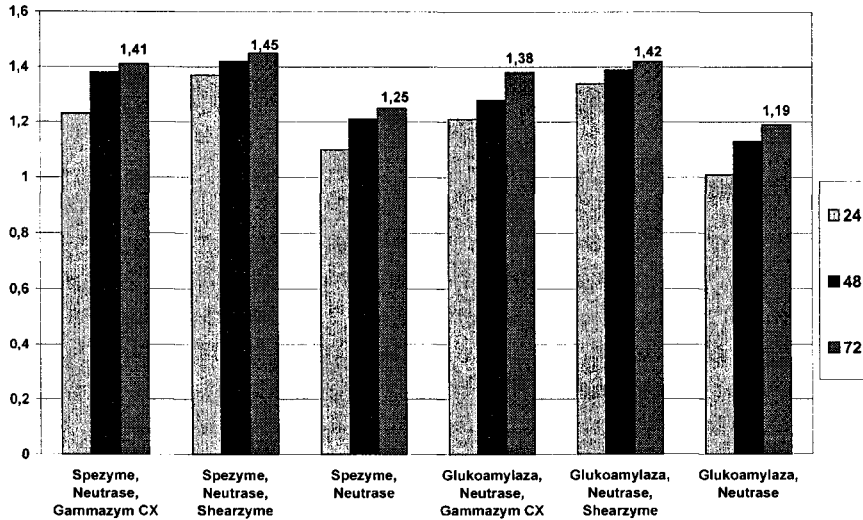


Diagram 2. Filtration rate of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with an additive of various cellulolytic preparations and of a proteolytic preparation.

X: Duration of hydrolysis [h]; Y: Filtration rate [$\text{cm}^3/\text{m}^2\text{s}$]

Due to the decomposition of amylase-lipid complexes, lysophospholipase contained in Spezyme preparation improves filtration ability of wheat glucose hydrolyzates.

The hydrolyzates obtained using in the process of saccharification Spezyme preparation with the addition of xylanase Shearzyme and proteinase, were characterised by the best filtration ability – $1.45 \text{ cm}^3/\text{m}^2\text{s}$. For the same system with the use of glucoamylase AMG 300L the hydrolyzates were filtered at a slower rate – $1.42 \text{ cm}^3/\text{m}^2\text{s}$.

Filtration ability of wheat glucose hydrolyzates is shown in Graph 2.

The application of both saccharifying preparations, cellulolytic enzymes and proteinase makes it possible to obtain glucose hydrolyzates which are less coloured and more transparent than those obtained without the addition of the enzymatic preparations.

The best appeared to be the system in which glucoamylase with lysophospholipase (Spezyme GA 300W), xylanase Shearzyme 500L and proteinase Neutrase 0.5L were used; the colour factor was then 206 and transparency factor – 122. For hydrolyzates obtained with the use of glucoamylase AMG 300L these factors were worse: 297 and 115, respectively.

The colour and transparency factors are shown in Graphs 3 and 4.

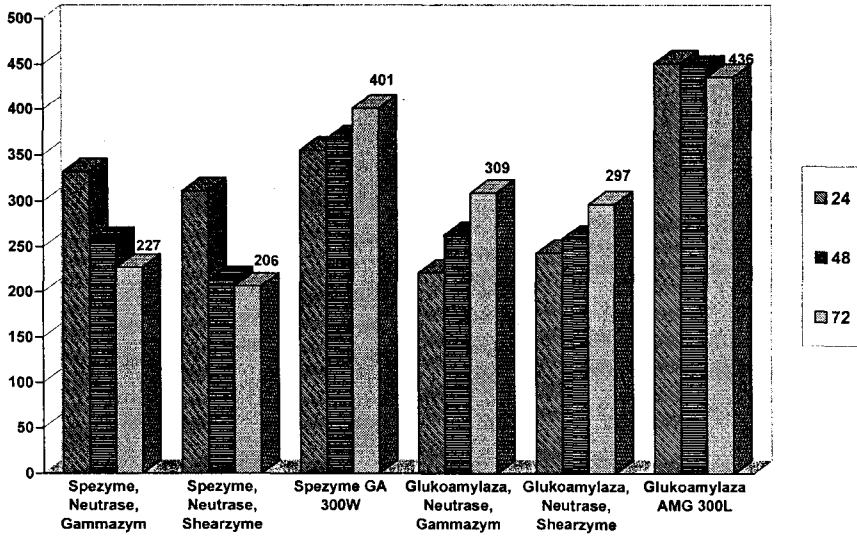


Diagram 3. The colour factor of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L with an additive of various cellulolytic preparations and of a proteolytic preparation.

X: Duration of hydrolysis [h]; Y: Colour factor

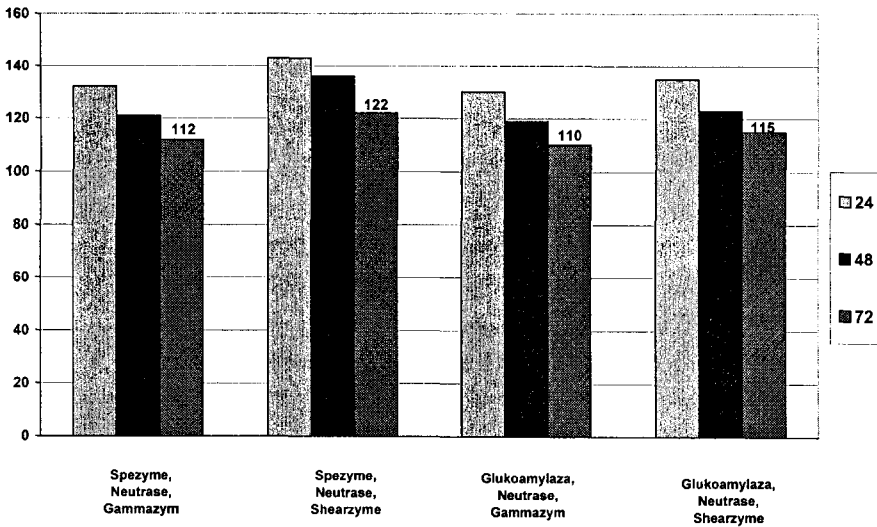


Diagram 4. The transparency factor of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with an additive of various cellulolytic preparations and of a proteolytic preparation.

X: Duration of hydrolysis [h]; Y: Transparency factor

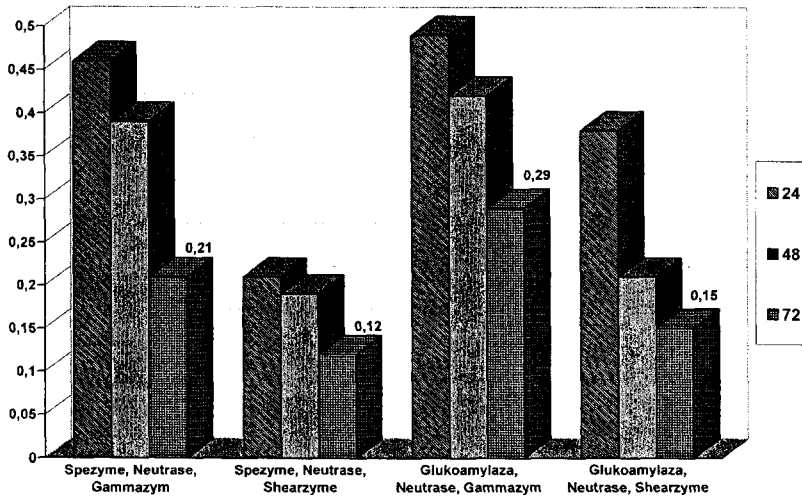


Diagram 5. The protein content in a glucose hydrolyzate obtained in the process of saccharification, using Spezyme preparation and glucoamylase AMG 300L, as well as Neutrase, a proteolytic preparation. Protein content: liquefied hydrolyzate-0,79% d.m.
X: Duration of hydrolysis [h]; Y: Protein content [%s.s.]

Investigations of protein content in glucose hydrolyzates (Graph 5) showed that much less protein is transferred to the solution when in the process of saccharification, beside glucoamylase and lysofosfolipase (Spezyme GA 300W), cellulolytic preparations and proteinase are used, than in the case of application of glucoamylase preparation AMG 300L with the above mentioned assisting enzymes.

Conclusions

1. Using cellulolytic enzymes and proteinase, simultaneously with saccharificating preparations, in the process of saccharification of wheat starch has an affect of an additional increase of glucose hydrolyzates.
2. Better filtration ability is revealed by glucose hydrolyzates obtained in the presence of glucoamylase and lysophospholipase (Spezyme) with the addition of cellulolytic preparations and proteinase, than by those obtained when glucoamylase AMG 300L is used with these enzymes.
3. The application of Spezyme and, additionally, cellulolytic enzymes and proteinase in the process of saccharification results in formation of glucose hydrolyzates which are less coloured and more transparent than in the case when these enzymes are used jointly with glucoamylase AMG 300L.
4. The best physical and chemical properties of glucose hydrolyzates, i.e. colour, transparency, filtration ability and the highest reductivity are obtained using for

saccharification both Spezyme, the enzymatic preparation of xylanase Shearzyme 500L and proteinase Neutrase 0.5L.

REFERENCES

- [1] Acker L., Schmitz H. J.: Über die Lipide der Weizenstärke. III. Mit. Die übrigen Lipide der Weizenstärke sowie die Lipide anderer Stärkearten. *Starch/Stärke*, **19**, 1967, 275.
- [2] Bowler P., Towersey P.J., Galliard T.: Some effects of the minor components of wheat starch on glucose syrup production. *Starch/ Stärke*, **37**, 1985, 351.
- [3] Chmiel A.: *Biotechnology: Microbiological and Biochemical Fundamentals*. PWN, Warszawa 1994, 37-39 (in Polish).
- [4] Konieczny-Janda G., Richter G.: Progress in the enzymatic saccharification on wheat starch. *Starch/Stärke*, **43**, 1991, 308.
- [5] Lionel A., Ghiskin W.: Carbohydrate refining process and enzyme compositions suitable for use therein. BE Pat. 86 307449.5, 1986.
- [6] McCleary B.V., Gibson T.S., Allen H., Gams T.C.: Enzymic hydrolysis and industrial importance of barley β -glucans and wheat flour pentosans. *Starch/ Stärke*, **38**, 1986, 433.
- [7] Polish Standard: Starch hydrolyzates. Methods of investigations. PN-78/A-74701 (in Polish).
- [8] Słomińska L.: Starch hydrolyzates obtained from corn products. *Przemysł Spoż.*, **51**, 1997, 9 (in Polish).
- [9] Swinkels J.J.M.: Composition and properties of commercial native starches. *Starch/Stärke* **37**, 1985, 1.
- [10] Świechowski Cz.: Technical analysis in food industry. WPLiS, Warszawa 1968, 350 (in Polish).
- [11] Tegge G., Richter G.: Zur enzymatischen Hydrolyse verschiedener Stärkearten. *Starch/Stärke* **38**, 1986, 329.
- [12] Trzmieł T.: Properties of enzymes and their applications. *Przemysł Spoż.*, **9**, 1995, 338 (in Polish).

WPLYW WARUNKÓW PROCESU ENZYMATYCZNEJ HYDROLIZY NA WŁAŚCIWOŚCI HYDROLIZATÓW SKROBI PSZENNEJ

Streszczenie

W przedstawionej pracy otrzymano hydrolizaty glukozowe działając na upłynnioną za pomocą bakteryjnej α -amylazy skrobię pszenną preparatem enzymatycznym Spezyme GA 300W, w skład którego wchodzi glukoamylaza i lizofosfolipaza oraz preparatem glukoamylazy Amyloglucosidase AMG 300L. Jednocześnie z enzymami scukrzającymi stosowano enzymy celulolityczne Gammazym CX 4000L i Shearzyme 500L oraz proteinazę Neutrase 0,5L.

Najlepsze właściwości fizykochemiczne hydrolizatów glukozowych, tj. barwę, przezroczystość, zdolność filtracji i najwyższą redukcijność uzyskuje się, stosując w procesie scukrzania łącznie z preparatem Spezyme preparat ksylanazy Shearzyme 500L i proteinazy Neutrase 0,5L. ☒