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THE ROLE OF ENZYMES ACCOMPANYING GLUCOAMYLASE IN THE PROCESS OF ENZYMATIC SACCHARIFICATION OF STARCH

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Key words: starch saccharyfying, glucoamylase, α-amylase, transglucosylase action, enzymatic reversion.

The effect of accompanying amylolytic enzymes on the starch saccharification process using a glucoamylase preparation, was investigated. The real effect of transglucosylase on the glucose yield was determined. On the other hand, no action of α-amylase on this preparation was observed.

A phenomenon of a negative character, known under the term of reversion [1, 8, 11] takes place in the two-stage enzymatic starch hydrolysis process. Responsibility for enzymatic reversion is ascribed to transglucosilase, an enzyme present in the majority of amylolytic preparations of the mould origin. In more recent studies the transglycolytic functions are more and more frequently ascribed to α-glucosidase, in accordance with the hypothesis that these two enzymes constitute a protein complex with sub-units of different active centres and, consequently, different catalytic functions [9].

Among the oligosugars of the isomaltose and maltose series, produced by reversion, Laszlo and Bartfay [2, 12] distinct out isomaltose as the most intensively represented sugar. As a result of reversion the amount of glucose produced is reduced, and the products obtained impede the filtration and crystallisation of syrups [2, 6, 20].

Practicians think, that also the α-amylase present in the complex glucogenic preparations plays a negative role in the process of the enzymatic saccharification of starch. This is supposed to be due to the fact that α-amylase produces dextrines of short chains, and maltose, which — being less suceptible to the action of glucoamylase — constitutes a parent substance for transglycolytic enzymes.

It seemed, therefore, worth while to investigate to what an extent the enzymes accompanying glucoamylase — particularly α-amylase and transglucosylase — present in enzymatic preparations of microbiological origin, affect the result of starch hydrolysis. Investigations were carried out with the use of model enzyme systems and the effect these systems was expressed in terms of the amount of glucose produced from starch (DX).

MATERIALS AND METHODS

Commercial preparations from the Danish firm of NOVO INDUSTRI A/S: Amyloglocosidase NOVO 150 and Bacterial Amylase NOVO were used for testing. These preparations were selected from a number of various preparations available, taking into account their high activity, very low content of impurities other enzymes and a high thermostability under the conditions of the enzymatic hydrolysis of starch. In the case of glucoamylase preparation, the high degree of saccharification of starch paste obtained was also taken into account.

The transglucosylase (α-glucosidase) preparation was obtained by the separation of proteins contained in a complex enzymatic preparation from Aspergillus awamori (AA_{72}). The separation method described by Dobrolinska [7], based on the fractionation of material in a column with DEAE-cellulose using 0.025 M of Sörensens buffer of pH 8.0.

Hydrolysis of 33% suspension of starch was carried out by the enzymatic two-stage system according to the method given by the firm of NOVO for its enzymatic preparations [13].

Liquefaction was carried out using a preparation of bacterial α-amylase, and saccharification — with the use of glucoamylase AMG NOVO 150. The analysis of the syrup after hydrolysis was made basing on the Polish Standard PN-70/a-74701 [16] determining the quantity of reducing sugars (DE) and glucose (DX) by the Steinhoff method [5].

Paper chromatography of sugars was carried out by the ascending technique on Whatmann 1 paper, with twice repeated development in the butanolethanol:water system of solvents at a ratio of 3:2:1. Trevelyan's test with silver nitrate and the Buchanan-Savage [14, 19] agent were used for the development.

RESULTS

Tests were carried out in four model enzyme systems: 1) glucoamylase, 2) glucoamylase + various quantities of α-amylase, 3) glucoamylase + various quantities of transglucosylase, and 4) glucoamylase + a constant amount of transglucosylase + various amounts of α-amylase.

Table 1 shows the results of starch saccharification with a glucoamylase preparation, NOVO 150, conducted in standard conditions. It should be stated that for this type of preparation better saccharification results have already been obtained [17]; nevertheless, also the results presented in Table 1 are satisfactory.

Table 1. Two stage enzymic starch hydrolysis using glucoamylase AMG NOVO 150 preparation (standard conditions)

Time of hydrolysis (h)	GA units/1 g dry mass of starch	Reducing sugars DE	Glucose DX
0	—	20.3	0.0
48	30	95.3	94.1
	40	95.7	92.2
72	30	96.5	93.4
	40	97.6	93.8

In the next experiment the effect of α-amylase added to the gelatinized starch while it was being saccharified by glucoamylase was investigated. α-amylase was added in the amounts of 0-500 SKB units per 11 g of dry substance of starch being saccharified. The results of this experiment are shown in Fig. 1. As can be seen, the addition of α-amylase has no particular effect on the result of saccharification.

The results of saccharyfying gelatinized starch with glucoamylase in the presence of transglucosylase (α-glucosidase) added in varying quantities, are shown in Fig. 2. The transglucosylase (α-glucosidase) preparation was added to the starch being saccharified in a amount of 11 to 95 TG units

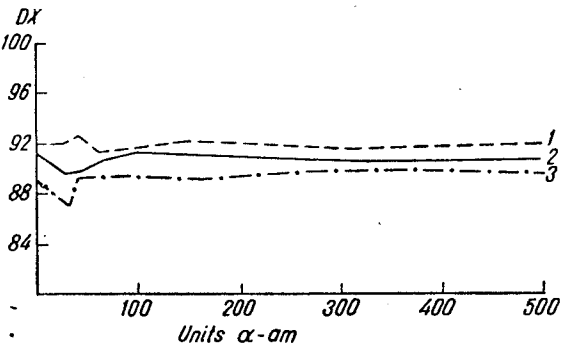

Fig. 1. The effect of α-amylase on the starch paste saccharyfying process using glucoamylase NOVO 150 preparation (DX value); Description: time of hydrolysis process — 1 — 72 h, 2 — 48 h, 3 — 24 h

per 11 g of dry substance of starch. It should be stated that the amount of glucose produced depends mainly — if not exclusively — on the amount of transglucosylase added. The course of the enzymatic saccharification of syrups with the addition of varying amounts of transglucosylase is shown in Fig. 3. It has been found that for each dose of enzyme the

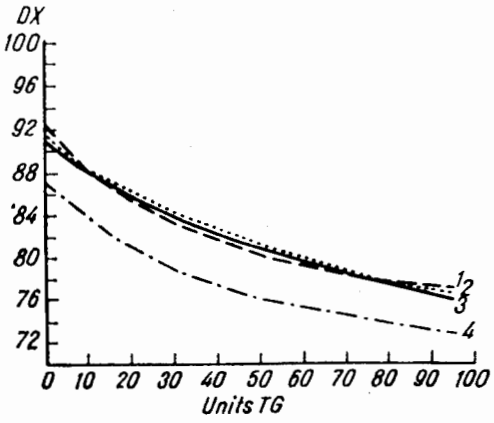

Fig. 2. The effect of the various amounts of transglucosylase on the result of starch hydrolysis using glucoamylase NOVO 150 preparation (DX value); Description: time of hydrolysis process — 1 — 72 h, 2 — 96 h, 3 — 48 h, 4 — 24 h

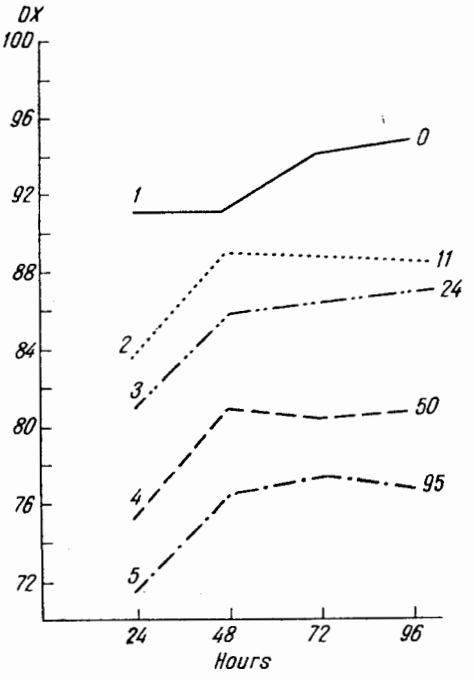

Fig. 3. The relationship between DX and time of the enzymatic hydrolysis. The testes with various amounts of transglucosylase; Description: 1 — 0 units TG, 2 — 11 units TG, 3 — 24 units TG, 4 — 50 units TG, 5 — 95 units TG

concentration of glucose (DX) becomes constant already after 48 hours of saccharification, and it does not change any more in time. Most probably this is so because a equilibrium between the processes of hydrolysis and resynthesis has been established. On the other hand, a slow but continuous growth of the DX value is observed in the case of syrups being saccharified in the standard way (curve „1" in Fig. 3).

It should be stated that the content of di- and tri-saccharides in syrups grows with the increase of the content of transglucosylase in them. To be sure that this was not a result of an incomplete hydrolysis of starch, the sugars present in the syrups were separated chromato-

graphically, and identified. Figs 4 and 5 show the chromatograms of syrups saccharified in the presence of varying quantities of transglucosylase, after 72 and 96 hours of hydrolysis. In these sugars the amount of isomaltose (B″) grows with the increasing content of enzyme investigated. The same can be said about the content of panose (C′). In tests with increased content of transglucosylase, also the tetrasaccharide (D) identified as dextrantriosylglucoset has been found to appear. Pazur and

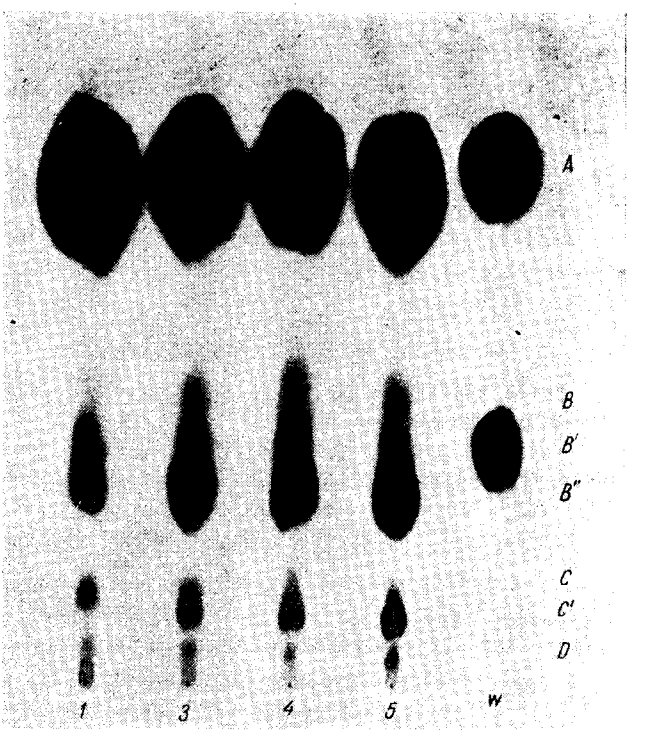

Fig. 4. Chromatogram of the starch sirups saccharifyied in the presence of the different amounts of transglucosylase (72 hours of hydrolysis); 1 — 0 units TG, 3 — 24 units TG, 4 — 50 units TG, 5 — 95 units TG, W — standard Gl+Ma; A — glucose, B — nigerose, B′ — maltose, B″ — isomaltose, C — maltotriose, C′ — panose, D — dextrantriosylglucose

French [15] have shown that it is formed when the glucosyl is transferred to panose. The test with diphenylamine was helpful in the identification of sugars with α-1.4 and α-1.6-glycoside bonds.

In the next experiment a model system corresponding, in general, to the composition of enzymes occuring in non-purified glucoamylase preparations, was used. Fig. 6 shows the course of starch saccharification with glucoamylase, in the presence of a constant amount of transglucosylase and varying doses of α-amylase. It was expected that in this case the effect of α-amylase would be marked with a distinct reduction of

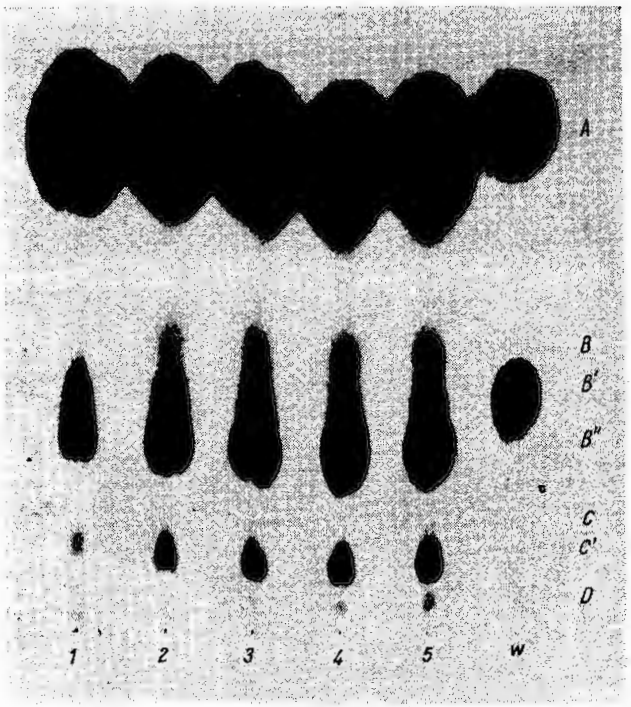

Fig. 5. Chromatogram of the starch sirups saccharifyied in the presence of the different amounts of transglucosylase (96 hours of hydrolysis); 1 — 0 units TG, 2 — 11 units TG, 3 — 24 units TG, 4 — 50 units TG, 5 — 95 units TG, W — standard Gl+Ma; A — glucose, B — nigerose, B′ — maltose, B″ — isomaltose, C — maltotriose, C′ — panose, D — dextrantriosylglucose

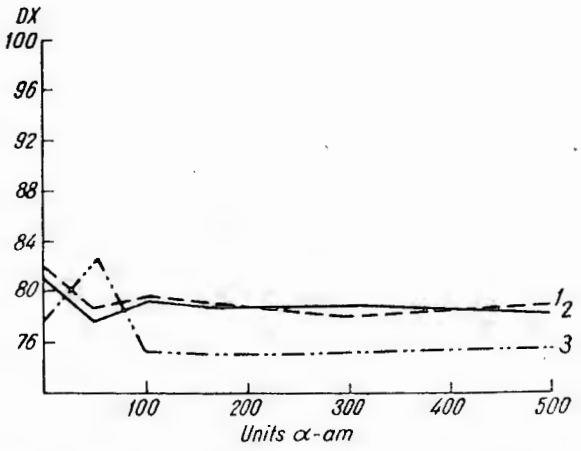

Fig. 6. The effect of α-amylase on the starch paste saccharyfying process using constant amounts of glucoamylase and transglucosylase preparations (DX value); Description: 1 — 72 h, 2 — 48 h, 3 — 24 h

glucose output, the higher the more of this enzyme is added to the syrup saccharified. The DX values obtained did not confirm that supposition. The results were similar to those obtained in the model "glucoamylase + transglucosylase" system with the enzyme content of approximately 90 TG units per 11 g of the dry starch substance. Thus, the conclusion could be repeated, that α-amylase has no effect on the starch saccharification results irrespective of how much of this enzyme has been added to the syrup.

DISCUSSION

The problem of investigation of the artificially composed model mixtures of amylolytic enzymes is connected with the problem of utilisation, as the producers of glucoamylase, of these fungi which in addition to glucoamylase produce other amylolytic enzymes. Practically it is therefore the problem of using, for the production of glucose, the complex, non-purified glucogenic preparations.

The investigations indicate that in addition to glucoamylase, a decisive effect on the output of the enzymatic hydrolysis is performed by transglucosylase, and that the latter should be made responsible for the incomplete output of glucose (DX), often reduced by as much as a few of more than a dozen per cent.

It is known that the process of transglucosylation is more intensive in the presence of high concentrations of the parent substance. Nevertheless, this process was also observed in a 1% solution of starch [3]. However, at low concentrations of starch saccharified, the hydrolytic action of both glucoamylase and α-glucosidase dominates, and the reactions of transfer do not affect the result of saccharification. Bendeckij, for example, does not notice any effect of tranusglucosylase on glucose output in case of a 4% solution of starch.

In order not to deviate from conditions under which the industrial glucose production process takes place, in this study a constant concentration of the starch solution approximately 33%, was used. In these conditions it was possible to foresee the intensified activity of transglucosylase. This has been confirmed by experiments with models. It results from these experiments that any quantity of transglucosylase present in the syrup being saccharified, reduces the output of glucose. The lowest content of enzyme used in model experimentation (11 units TG) results in a DX loss by some 5-6%, and the highest enzyme content (95 units TG) — by approximately 14%.

Many data indicate that the high concentration of the starch hydrolysis products (maltose and glucose) rather than the starch itself is the factor that stimulates the activity of transglucosylase.

In the experiments described in this paper the hypothesis about the negative role of α-amylase in the process of saccharification of starch to glucose, has not been confirmed. In systems where it occurs only with glucoamylase, and also in the model set where apart of glucoamylase it is accompanied by a certain constant amount of transglucosylase, α-amylase does not affect the result of saccharification. Thus, the views related to obtaining higher saccharification outputs by selective inactivation of this enzyme [10] should be verified. In the light of study reported in this paper, more essential is the inactivation of other glucosidases rather than the α-amylase alone.

The amount of α-amylase ussualy present in glucogenic fungal enzyme preparations does not decide about the result of the saccharification of starch. It is certain, however, that strains of fungi considered to be producers of glucoamylase should be completely free of the presence of transglucosylase. This is just the enzyme which should be made responsible for the incomplete, unsatisfactory starch saccharification degree.

CONCLUSIONS

1. In the process of the enzymatic saccharification of starch to glucose using non-purified preparations of glucoamylase, a phenomenon of enzymatic reversion is observed. This phenomenon is responsible for the lower output of glucose.
2. It has been found that only transglucosylase present in the enzymatic preparation used for saccharification can be responsible for enzymatic reversion.
3. The percentage output of glucose decreases nonlinearly with the growth of transglucosylase content.
4. α-amylase present in the sirup has no effect on the result of saccharification, irrespective of the amount of its content.
5. α-amylase does not stimulate the activity of transglucosilase, neither.

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ROLA ENZYMÓW TOWARZYSZĄCYCH GLUKOAMYLAZIE W PROCESIE ENZYMATYCZNEGO SCUKRZANIA SKROBI

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Streszczenie

Przebadano rolę obecności α-amylazy i transglukozylazy w czasie enzymatycznego scukrzania skrobi do glukozy. W tym celu sporządzono układy złożone z enzymów: glukoamylazy, α-amylazy i transglukozylazy. Scukrzanie upłynnionej skrobi prowadzono przy użyciu następujących układów enzymowych: 1) glukoamylazy (tab. 1), 2) glukoamylaza+α-amylaza — zmienne ilości (rys. 1), 3) glukoamylaza+transglukozylaza — zmienne ilości (rys. 2), 4) glukoamylaza+transglukozylaza+α-amylaza (zmienne ilości) (rys. 6).

Stwierdzono, że obecna w scukrzanym syropie, aktywna α-amylaza, towarzysząca glukoamylazie — wi lości od 0 do 500 jedn. SKB na 11 g s.s. skrobi, nie powoduje obniżenia wydajności glukozy, niezależnie od tego, czy została ona wprowadzona do scukrzanego syropu odrębnie (model 2, rys. 1), czy w towarzystwie transglukozylazy (model 4, rys. 6). Natomiast transglukozylaza odgrywa decydująco niekorzystną rolę w czasie enzymatycznego scukrzania obniżając wydajność procesu w wyniku dokonywanej resyntezy enzymatycznej (rys. 2). Preparat transglukozylazy dodany do scukrzanego syropu w ilości 11 do 95 jedn. TG na 11 g s.s. skrobi powoduje spadek DX odpowiednio od 5-6% do 14%.

Ujęto ilościowo zależności między stężeniem transglukozylazy, a nasileniem reakcji resyntezy enzymatycznej. Wykazano, że zwykle po 48 h scukrzania ustala się równowaga procesów resyntezy i hydrolizy, podczas gdy w syropach scukrzanych standardowo (krzywa „O jedn. TG" na rys. 3) obserwuje się powolny, ale ciągły wzrost wartości DX.

Rozdzielono chromatograficznie produkty resyntezy i zidentyfikowano je w syropach po 72 i 96 h scukrzania (rys. 4 i 5). Stwierdzono obecność izomaltozy, maltotriozy, panozy i dekstrantriozylglukozy.