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PRODUCTION OF HIGH SACCHARIFIED SYRUPS BY TWO-STAGE ENZYMATIC HYDROLYSIS OF POTATO STARCH

PART I THE EFFECT OF GLUCOAMYLASE CONCENTRATION ON REDUCING-SUGAR CONTENT INCREASE AT CONSTANT AND VARIABLE PH VALUES IN HYDROLYSIS OF STARCH

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Key words: α-amylase, glucoamylase, high saccharified syrups, potato starch hydrolysis.

High saccharified syrups (50-75DE) were obtained by liquefaction of potato starch with α-amylase and saccharification with glucoamylase. Considerably reduced concentrations of glucoamylase preparations were used in hydrolysates to make the process more economical. A part of the experiments concerned enzymatic dissolving and saccharification of starch at constant pH 6. This was performed to avoid accretion of salts, which would have developed in an environment with varying pH.

Highly saccharified syrups have a high content of reducing sugars in the range 50-70 DE (dextrose equivalent). There are different methods of preparation of the syrups. During acid-enzymatic hydrolysis of starch hydrochloric acid is used first at 142°C, followed by a preparation of amylase with *Aspergillus oryzae*. This treatment provides a syrup of 64 DE and glucose content above 35% [3, 5]. The second stage of the hydrolysis can also be performed with either sequential or simultaneous activities of two enzymes [4].

Conversion of starch can be effected by purified malt amylase and mould amylase, usually at 55°C. Miles Chemical Co. introduced a technology for manufacture of starch syrups involving acid hydrolysis of starch to arrive at 15-20 DE and then additional saccharification of the hyrolysate with a mould enzyme present in Dextrinase 80. The resultant syrup re-

ducing-sugar content is of the order of 67-69 DE, 40% glucose and 40% maltose, and it undergoes fermentation to a level of 80-82% [5]. Glucose content in starch syrups prepared by acid hydrolysis should not exceed 44% in order that the syrup does not crystallize [5].

The Röhm-Haas method for manufacture of syrups consists in substitution of acid hydrolysis with enzymatic liquefaction of starch. The method involves addition of dry or moist starch to water (70-90°C) containing an enzyme. The solution is stirred until all starch is liquefied. Then enzymatic saccharification of the solution is applied until a desired level of DE is reached [1, 2]. A positive aspect of this method is that it can be used at high — up to 75% d.s. (dry substance) — concentrazions of substrate, which reduces the demand for heating steam during condensation of hydrolysate. A negative side of it are difficulties in separation of impurities from hydrolysate by filtration or centrifuging [1].

To determine the effect of glucoamylase concentration on starch saccharification during two-stage enzymatic hydrolysis a series of experiments was carried out, as described in the following section of the paper.

THE EXPERIMENTAL PROCEDURES

Starch milk concentrate of 30% was supplemented with α-amylase preparations to liquefy and partially hydrolyze starch. Starch liquefaction was carried out under the conditions most favourable for activity of the applied enzymatic preparations. Starch milk at room temperature and pH 6.0 was supplement with 0.05% (in terms of dry starch) α-amylase Novo 264 preparation, or 0.15% α-amylase HT-1000 preparation at pH 7.0. While rapid stirring was continued, the temperature was brought up to 60°C, and then the temperature was still increased in by 1.5°C every minute until 85°C for the α-amylase Novo 264 preparation, or until 70°C for the α-amylase HT-1000 preparation. The hydrolysate at this temperature was continuously stirred for 1.5 hr. Subsequently, the temperature of the hydrolysate was reduced to 60°C and pH 4.5, which was optimally effective for glucoamylase, and then preparations of glucoamylase Novo 75 or Diazyme L-30 were added in gradually smaller quantities from the optimum concentration suggested by the manufacturer, including even the level of 1/10th of the concentrations. When further saccharification of starch was done with the Sumy-zyme glucoamylase preparation the concentration of the enzyme was similar but the temperature was reduced to 55°C and pH maintained at 5.2.

The saccharification was on for 48 hrs, the reaction time being counted from the moment of introduction of the glucoamylase preparation. In the

course of the process samples of the hydrolysate were monitored as to the contents of reducing sugar and apparent level of dry substance (°Bx).

In comparison with processes of enzymatic hydrolysis described in the literature, the present experiments did not involve thermic inactivation of enzyme following the dissolving of starch. This was done only after starch saccharification. This way during saccharification the α-amylase was still active and it co-worked with glucoamylase. After saccharification the starch hydrolysates were boiled over 10 minutes at 100°C. It was performed not only to inactivate the two enzymes but also to facilitate filtration of the hydrolysates (protein coagulation). Results of the experimental procedures are given in Tables 1, 2 and 3.

Table 1. The effect of quantity glucoamylase and reaction time on degree of saccharification (DE) of starch hydrolysates during the combined activity of α-amylase Novo 264 glucoamylase Novo 75. Liquefaction of starch at pH 6.0; saccharification at pH 4.5. Starch hydrolysate concentration after liquefaction: 30.4 °Bx; reducing sugar content — 31.7 DE

Hydrolysate	Quantity of the added glucoamylase preparation (% dry substance of hydrolysate)	Reducing sugar content (DE) after elapsed reaction times (hrs)					
		4.5	6.0	12.0	24.0	36.0	48.0
1	0,025	41.9	46.7	54.8	58.3	66.7	72.8
2	0.050	43.3	47.7	56.1	61.4	69.3	75.3
3	0.075	45.1	49.2	63.2	71.0	75.1	78.6
4	0.100	49.6	53.2	70.2	80.8	81.9	82.5
5	0.125	52.8	57.0	73.1	84.1	84.7	86.4
6	0.250	56.1	61.0	78.4	90.2	90.5	90.8

In contrast with the previous experiments the liquefaction of starch and saccharification of the hydrolysate in subsequent experiments were conducted at constant pH = 6.0, which was an intermediate value between the optimum pH levels for starch liquefaction with the α-amylase HT-1000 (pH = 7.0) and for saccharification of the starch hyrolisate with the Sumyzyme glucoamylase preparation (pH = 5.8). This provided a possibility to bypass the need to alkalize starch milk to bring pH to 7.0 and then acidify the solution of liquid starch to pH 5.2. Owing to this the content of salts in the hydrolysate solution did not increase.

Starch milk concentrated at 30% and pH 6.0 was supplemented with 0.15% α-amylase HT-1000 preparation, in terms of starch dry substance. The starch suspension in water was brought up to 70°C, at which starch dissolving was continued for 1.5 hr. Then, the temperature of hydrolisate

solution was reduced to 55°C, while pH was maintained at the same level. Next the hydrolysate was divided into six portions, each of which was supplemented varying amounts of the Sumy-zyme glucoamylase preparation: 0.05; 0.1; 0.2; 0.3; 0.4 and 0.5% in terms of hydrolysate dry substance. The hydrolysate saccharification was continued for 48 hrs at 55°C. At appropriate time intervals the reducing sugar (DE) content and the

DISCUSSION OF RESULTS.

Table 2. The effect of quantity of glucoamylase and reaction time on starch hydrolysate saccharification degree (DE) during the combined activity of α-amylase Novo 264 and glucoamylase Sumy-zyme. Liquefaction of starch at pH = 6.0, saccharification at pH = = 5.2. Concentration of starch hydrolysate after liquefaction — 30.2°Bx; reduction — 31.0 DE

Hydrolysate	Quantity of the added glucoamylase preparation (% dry substance of hydrolysate)	Reducing sugar content (DE) after elapsed reaction times (hrs)					
		4.5	6.0	12.0	24.0	36.0	48.0
1	0.05	42.8	47.4	56.1	61.0	67.2	71.4
2	0.10	44.3	56.7	67.5	73.6	75.0	75.9
3	0.15	52.3	64.1	71.0	75.5	78.1	80.0
4	0.20	61.0	68.2	75.3	78.2	82.7	85.2
5	0.25	69.7	72.1	79.4	82.3	86.6	89.0
6	0.50	73.2	82.0	88.5	92.3	93.9	94.7

Table 3. The effect of quantity of glucoamylase and reaction time on starch hydrolysate saccharification degree (DE) during the combined activity of α-amylase HT-1000 and glucoamylase Diazyme L-30. Liquefaction af starch at pH = 7.0; saccharification at pH = 4.5. Concentration of starch hydrolysate after liquefaction — 30.7°Bx; reduction — 30.2 DE

Hydrolysate	Quantity of the added glucoamylase preparation (% dry substance of hydrolysate)	Reducing sugar content (DE) after elapsed reaction times (hrs)					
		4.5	6.0	12.0	24.0	36.0	48.0
1	0.025	39.9	43.3	48.8	55.5	60.5	70.3
2	0.050	40.2	45.0	52.1	57.8	64.3	74.9
3	0.075	41.5	46.6	54.2	61.8	67.5	78.1
4	0.100	43.7	48.7	58.1	66.6	73.3	82.7
5	0.125	46.7	50.9	63.2	72.7	79.1	85.1
6	0.250	53.2	58.1	76.1	86.0	89.5	92.4

apparent dry substance (°Bx) content were determined. Results of the experimental procedures are given in Table 4.

The experiments proved that by treating starch suspension in water, first with α-amylase and then with α-amylase and glucoamylase simultaneously, highly saccharified syrups can be obtained. The reducing sugar content in syrups obtained this way depends on the kinds of enzymatic preparations used in the process, but above all it depends on the glucoamylase concentration and the hydrolysis time.

Table 4. The effect of quantity of glucoamylase and reaction time on degree of saccharification (DE) of starch hydrolysates during the combined activity of α-amylase HT-1000 and glucoamylase Sumy-zyme at constant pH = 6.0 during the entire process of starch hydrolysis; starch hydrolysate concentration after liquefaction — 30.5°Bx; reduction — 25.5 DE

Hydrolysate	Quantity of the added glucoamylase preparation (% dry substance of hydrolysate)	Reducing sugar content (DE) after elapsed reaction times (hrs)					
		4.5	6.0	12.0	24.0	36.0	48.0
1	0.05	37.3	42.1	49.0	53.9	62.0	68.2
2	0.10	41.6	48.4	57.3	64.2	69.3	73.5
3	0.15	44.4	54.0	63.2	71.3	76.0	81.2
4	0.20	47.2	59.3	67.1	75.5	83.5	90.9
5	0.25	53.9	62.6	72.9	82.6	89.5	94.8
6	0.50	58.5	66.2	78.8	87.9	92.0	95.5

The highly saccharified syrups, with their reducing sugar levels above 50DE, may be obtained in the course of the two-stage enzymatic hydrolysis with the use of ten-times lower than the suggested by manufacturers of enzymes concentrations of glucoamylase, as the experiments confirmed it. Already at the minimum dose of 0.025% glucoamylase Novo 75, in terms of starch dry substance, at the optimum pH=4.5 and during 12 to 24 hrs of hydrolysis, the degree of saccharification may reach 55-60 DE (Table 1, Line 1). Similar effects with the Sumy-zyme glucoamylase are obtained at 0.05% dose of the enzymatic preparation and the optimum pH=5.2 (Table 2, Line 1). The above small doses of glucoamylase make it possible to reach the degree of saccharification of the order of 65-75 DE but it requires further extension of the enzymatic hydrolysis (Tables 1 and 2, Line 1).

Highly saccharified syrups with about 55 DE in reducing sugar may be obtained within a much shorter time, e.g. 6 hrs, but it calls for a higher concentration of the glucoamylase preparation, such as ca 0.10% in terms of the dry substance of substrate (Table 1, Line 4 and Table 2, Line 2).

The preparation of glucose solutions with their levels of reducing sugar exceeding 90 DE is possible over a period of 24 hrs of hydrolysis but it calls for a ten-times higher dose of enzyme: 0.25% glucoamylase Novo 75 or Diazyme L-30, or possibly 0.50% glucoamylase Sumy-zyme (Tables 1, 2, 3, Line 6).

During the first part of the experiments highly saccharified syrups then were obtained, including the cases when minimum doses of enzymes were added. During the enzymatic hydrolysis various levels of pH, optimal for particular enzymatic preparations, were used. For example, during the dissolving of starch pH was at 6.0 for α-amylase Novo 264, and then pH=5.2 for glucoamylase Sumy-zyme which in the second stage of saccharifies the hydrolysate by coworking with α-amylase present in the solution (Table 2).

In the second part of the experiments, when the two-stage enzymatic hydrolysis was applied at a constant value of pH=6.0 (which was intermediate in relation to pH optimum values of the two enzymes of α-amylase HT-1000 and glucoamylase Sumy-zyme), highly saccharified syrups were also obtained but their reducing sugar levels were generally somewhat lower (Table 4).

A comparison of the results indicates that during starch hydrolysis at variable and constant pH the greatest differences in the reducing sugar contents can be observed for shorter periods of hydrolysis (e.g. 6 hrs). Extension of hydrolysis time to 48 hrs brings in a reduction of these differences (Table 2 and Table 4, Lines 1-6).

An unquestionable economic effect of the experiments was the indication that very small dosages of glucoamylase preparations can be applied to obtain highly saccharified syrups, which is highly significant for technological production of the syrups considering the high prices of the enzymatic preparations.

The generally somewhat lower reducing sugar contents in syrups obtained while pH is kept constant (e.g. 6.0) throughout the entire two-stage enzymatic hydrolysis process is made up for by the profits following from a reduction of salt content in syrups because there is no need to acidify and neutralize the hydrolysates to pH levels indispensable for particular stages of the hydrolysis and for the final product.

CONCLUSIONS

1. Highly saccharified syrups may be obtained from starch by means of two-stage enzymatic hydrolysis at glucoamylase concentrations ten times lower than it is suggested by the enzyme manufacturers.

2. Lower glucoamylase concentrations have effects on reducing sugar levels in starch hydralysates. In order to obtain highly saccharified syrups at lower levels of glucoamylase the time of hydrolysis must be appropriately longer.

3. Highly saccharified syrups may be obtained at constant pH, that is, identical for the two stages of the enzymatic hydrolysis. The reducing sugar levels are then somewhat lower than the DE values in the hydrolysates obtained at pH levels optimal for α-amylase and glucoamylase activity.

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WYTWARZANIE SYROPÓW WYSOKOSCUKRZONYCH PRZEZ DWUSTOPNIOWĄ HYDROLIZĘ ENZYMATYCZNĄ SKROBI ZIEMNIACZANEJ

CZĘŚĆ I. WPŁYW STĘŻENIA GLUKOAMYLAZY NA PRZYROST ZAWARTOŚCI CUKRÓW REDUKUJĄCYCH PRZY STAŁEJ I ZMIENNEJ WARTOŚCI pH W PROCESIE HYDROLIZY SKROBI

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Streszczenie

Syropy wysokoscukrzone o redukcyjności 50-75 DE otrzymywano metodą enzymatycznej hydrolizy skrobi. Do upłynniania skrobi i częściowego jej scukrzania stosowano preparaty bakteryjnej α-amylazy. W celu uzyskania dużej zawartości cukrów redukujących w syropach wysokoscukrzonych stosowano do dalszej hydrolizy preparaty glukoamylazy, w ilościach kilkakrotnie mniejszych od ogólnie używanych i zalecanych przez producentów tych preparatów. Miało to na celu poprawienie efektywności ekonomicznej wytwarzania syropów wysokoscukrzonych. Stosując minimalną dawkę preparatu glukoamylazy Novo 75, wynoszącą 0,025% preparatu licząc

na s.s. substratu, uzyskiwano stopień scukrzenia hydrolizatów w granicach 55 do 60 DE. Proces scukrzania prowadzono przy optymalnej wartości pH, w czasie 12-24 godzin. Syropy wysokoscukrzone o zawartości cukrów redukujących ok. 55 DE można również otrzymać w znacznie krótszym czasie np. 6 godz., ale przy wyższym stężeniu preparatów glukoamylazy, tj. ok. 0,10% s.s. substratu. W pierwszej części doświadczeń uzyskiwano zatem syropy wysokoscukrzone, stosując różne wartości pH optymalne dla działania poszczególnych preparatów enzymatycznych np. pH 6,0 dla α-amylazy Novo 264, w fazie rozpuszczania skrobi i pH 5,2 dla glukoamylazy Sumyzyme w fazie scukrzania. Scukrzanie hydrolizatów prowadzono przy współdziałaniu glukoamylazy z obecną w roztworze nie zdezaktywowaną α-amylazą. W drugiej części doświadczeń, stosując dwustopniową enzymatyczną hydrolizę przy jednej tylko niezmiennej wartości pH = 6,0 (pośredniej w stosunku do optymalnej wartości pH 7,0 dla działania α-amylazy HT-1000 i optymalnej wartości pH 5,2 dla działania glukoamylazy Sumy-zyme), otrzymywano również syropy wysokoscukrzone o niewiele mniejszych zawartościach cukrów redukujących. Nieco niższe wartości DE syropów uzyskiwanych przy utrzymywaniu stałej wartości pH np. 6,0 w całym procesie dwustopniowej hydrolizy enzymatycznej rekompensuje korzystnie mniejsza zawartość soli w syropach, ponieważ nie występuje wówczas konieczność na przemian zakwaszania i zobojętniania hydrolizatów do wartości pH niezbędnych dla poszczególnych etapów hydrolizy oraz odpowiednich dla końcowego produktu.