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THE PERIODIC ISOMERIZATION OF GLUCOSE IN STARCH HYDROLYSATES, USING A SOLUBLE AND INSOLUBLE PREPARATION OF GLUCOSE ISOMERASE

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Key words: glucose isomerase, starch hydrolysates, periodic isomerisation, hydrol, confectionery syrup, glucose solution

Products of starch hydrolysates such as crystalline glucose, glucose solution for second crop crystallization, hydrol and confectionery syrup were subjected to the action of a soluble or insoluble preparation of glucose isomerase. The insoluble preparation was utilized many times in a series of consecutive stages of isomerization.

The question of finding sweeteners which could replace saccharose becomes more and more actual in the whole world. A special role is played by fructose the sweetness of which is about 1.3 times higher than that of saccharose [3, 4]. Owing to this fact, it can be used in smaller quantities than saccharose. Contrary to fructose, glucose is less suitable as a sweetening agent because its sweetness constitutes only about 0.7 of the saccharose sweetness [4, 12]. If, however, half of the glucose present in the glucose solution is converted into fructose, then the average sweetness of the solution will correspond to that of the saccharose solution with the same concentration. Such syrup is obtained by an enzymatic isomerization of glucose into fructose, using high-purity glucose solution, corresponding to 96-98 DE and meeting the requirements set by isomerase being the enzyme used in this process. There are many data in literature concerning the enzymatic isomerization of high-purity solution. Such substrate is, however, rather expensive and makes the discussed process less profitable from the economic point of view. Tests aimed at an isomerization of syrups derived from glucose crystallization (intermediate products of starch hydrolysis) were carried out in the Netherlands. The solutions under test were constituted by products somewhere between a glucose solution for second crystallization and hydrol (average DE = 85

and ash content in DS about 1%) [11]. In Poland, studies were conducted on the enzymatic isomerization of hydrol with different degrees of purity.

The aim of the present work was to study the enzymatic isomerization of cheaper substrates such as intermediate products formed during glucose crystallization, that is, second crop glucose solution, before the second crystallization and the last post-crystallization syrup called hydrol or confectionery syrup.

MATERIALS AND METHODS

The subject of the studies was a periodic isomerization of glucose in various products of starch hydrolysis. The single-use soluble preparation of glucose isomerase and repeated-use insoluble preparation of glucose isomerase, were utilized. Optisweet P from Miles Cali-Chemie (US), with an activity of 3600 TGIU/g, obtained from Streptomyces albus strain [5] was treated as a soluble preparation of glucose isomerase. The insoluble preparation of glucose isomerase, used in the experiments, was constituted by Sweetzyme — A, obtained from the Bacillus species strain [9].

In the experiments, the following substrates were used:

— solution of raw, i.e. non-purified hydrol, containing residues of wash syrup (from washing glucose crystals in a centrifuge), from the Potato Industry Plant "Łomża" (concentration of hydrol 60% DS), with a purity corresponding to 75 DE; containing 1.35% minerals (ash) in dry solids, including 0.075% calcium, 0.24% protein substances in dry solids,

— solution of hydrol as above, purified by a complete ionexchange (cation and anion exchangers) with 76 DE and ash and protein level lower than 0.01% in dry solids, with a practically completely eliminated calcium content (2 ppm),

— solution of hydrol as above, purified with ion exchangers and additionally saccharified with the use of gluco-amylase (0.12% Amyloglucosidase Novo 150 in dry solids to reductivity 83 DE; conditions of hydrolysis: pH = 4.5, temperature 60°C, 72 h),

— solution of hydrol without the addition of wash syrup from "Łomża" Potato Industry Plant, with reductivity 68 DE and concentration 60% DS, purified with the use of activated carbon and ion exchangers,

— dense glucose solution, destined for second crystallization with concentration 71% DS and reductivity 84 DE, purified with activated carbon and ion exchangers,

— solution of confectionery syrup from Potato Industry Plant "Luboń" (concentration of syrup 81% DS and 40.1 DE), purified with activated carbon and ion exchangers,

— solution of crystallic glucose (monohydrate with 90% DS and reductivity 99.9 DE) from "Łomża" Potato Industry Plant, purified with ion exchangers.

Since calcium is an inhibitor of the enzymatic isomerization process, it was completly eliminated together with other ions by the method of ion exchange (cation and anion exchanger); the respectiwe process was controlled by conductantce measurements. The conductance of ionized solutions should be lower than 10 μS [8].

The carbohydrate composition of substrate solutions was determined by chromatography (Whatman paper 1, developing 48 h with the following solution: n-propanol:water:ethyl acetate ratio 6:3:1; development according to Buchan-Savage [2]. The content of dextrins in confectionery syrup was determined by precipitation with ethanol. The amount of fructose formed during the process of isomerization was determined by polarometrycally from the difference in rotation of substrate solutions before and after isomerization [6] and controlled according to the chromatographic method of Buchan-Savage [2] or using a resorcine developer [10]. The staining of solutions was determined by measurements of the absorbance of substrates and isomerization products with 40% DS, at a 1 cm thick layer and light wave length $\lambda = 420$ nm.

EXPERIMENTS

In the first series of experiments, the following hydrol solutions were enzymatically isomerized: a) raw, b) purified and c) saccharified after preliminary treatment (purification). The chromatographic determination of the carbohydrate content of the examined hydrol solution is presented in Table 1. The periodic isomerization of hydrol solutions with a concentration of 40% DS was conducted at 74°C and pH adjusted to 6.5, for about 24 h, using a dose of soluble product of glucose isomerase — Optisweet-P corresponding to 20 TGIU/g of glucose and with the addition of activators: 2 g $MgSO_4 \cdot 7\,H_2O$ and 0.2 g $CaCl_2 \cdot 6\,H_2O$ for 1 dm^3

Table 1. Carbohydrate composition of the examined hydrol solutions before isomerization

Hydrol	DE	Content in dry solids (%)			
		glucose	maltose	isomaltose	maltotriose
Raw	75	64	10.7	13.8	10
Purified with ion exchangers	76	65	11	14	10
Purified and saccharified with glucoamylase	83	79	1	10	10

Table 2. Isomerization of glucose in hydrol solutions with the use of soluble preparation Optisweet P. (Temperature 74°C, pH = 6.5: concentration of substrate 40% DS; dose of enzyme 20 TGIU/g glucose)

The examined solution	Content of glucose before isomerization (% DS)	Time of reaction (h)	Absorbance of the solution (E)		Content of fructose produced (% DS)	Degree of isomerization (ID %)
			before isomerization	after isomerization		
Raw hydrol 75 DE	64	23.5	0.405	0.830	20.8	32.5
Purified hydrol 76 DE	65	24.5	0.050	0.450	28.3	43.5
Hydrol purified and saccharified with glucoamylase, 83 DE	79	24.5	0.150	1.10	39.5	50.0
Crystallic glucose, 99.9 DE	99.8	23	0.007	0.220	51.4	51.5

Table 3. Carbohydrate composition of isomerization substrates purified with activated carbon and ion exchangers

Substrate	DE	Percentage in dry solids									
		glucose	maltose	isomaltose	malto-triose	isomalto-triose	malto-tetrose	malto-pentose	malto-hexose	malto-heptose	dextrines
Crystallic glucose	99.9	99.8	traces	0	0	0	0	0	0	0	0
Hydrol	68	53	12	23	12	0	0	0	0	0	0
Glucose solution for second crystallization	84	76	6	12	6	0	0	0	0	0	0
Confectionery syrup	40,1	21	18	9	12	4	8	5	5	4	14

Table 4. Isomerization of glucose in a pure solution of glucose with DE = 99.9 with use of insoluble preparation Sweetzyme A, utilized 14 times (temp. 65°C, pH = 7.0; concn. of substrate 40% in DS, dose of enzyme in the first batch 10 GINU/g glucose)

Process of isomerization		Absorbance (E) 40% solution	Content of fructose produced in % DS	Degree of isomerization, ID in %
No. of batch	Time of isomerization (h)			
1	0	0.008	0	0
	24	0.135	45.0	45.1
2	0	0.010	2.1	2.1
	24	0.100	44.8	44.9
3	0	0.012	2.4	2.4
	24	0.070	44.1	44.2
4	0	0.013	1.6	1.6
	24	0.054	43.6	43.7
5	0	0.015	1.8	1.8
	24	0.044	42.7	42.8
6	0	0.014	1.9	1.9
	24	0.039	41.4	41.5
7	0	0.014	1.6	1.6
	24	0.035	39.6	39.7
8	0	0.012	1.8	1.8
	24	0.031	37.3	37.4
9	0	0.012	2.0	2.0
	24	0.028	35.0	35.4
10	0	0.011	2.4	2.4
	24	0.025	33.3	33.4
11	0	0.010	2.1	2.1
	24	0.022	31.0	31.1
12	0	0.010	1.8	1.8
	24	0.019	28.9	29.0
13	0	0.009	1.6	1.6
	24	0.015	26.7	26.8
14	0	0.008	1.7	1.7
	24	0.013	24.75	24.8

of 40% substrate [5]. Parallelly, under the same conditions, the isomerization in the ion exchange solution of crystallic glucose with 99.99 DE, was carried out. The results of the studies on the isomerization process of the substrates examined are given in Table 2.

In further series of the experiments, the periodic (batch) enzyme isomerization covered the following solutions: a) purified solutions of

Table 5. Isomerization of glucose in glucose solution for the second crystallization (purified with ion exchange resins), DE = 84 and 76% of glucose level with the use of insoluble preparation Sweetzyme A, utilized in 14 batches of isomerization (temp. 65°C, pH = 7.0; concn. of juice solution 40% in DS, dose of enzyme in the first batch 10 GINU/g d. s. carbohydrate)

Process of isomerization		Absorbance (E) 40% solution	Content of fructose produced (% DS)	Degree of isomerization ID in %
No. of batch	Time of isomerization (h)			
1	0	0.020	0	0
	24	0.236	33.0	43.4
2	0	0.030	1.35	1.8
	24	0.155	32.9	43.3
3	0	0.031	1.45	1.9
	24	0.110	32.3	42.5
4	0	0.029	1.2	1.6
	24	0.095	32.0	42.1
5	0	0.033	1.5	2.0
	24	0.085	31.3	41.2
6	0	0.035	1.8	2.4
	24	0.081	30.3	39.9
7	0	0.033	1.6	2.1
	24	0.080	29.0	38.2
8	0	0.029	1.35	1.8
	24	0.078	27.4	36.1
9	0	0.026	1.3	1.7
	24	0.075	25.8	33.9
10	0	0.026	1.45	1.9
	24	0.075	24.3	32.0
11	0	0.025	1.6	2.1
	24	0.070	22.7	29.9
12	0	0.020	1.2	1.6
	24	0.071	21.1	27.8
13	0	0.021	1.35	1.8
	24	0.068	19.8	26.0
14	0	0.019	1.5	2.0
	24	0.065	18.2	23.9

hydrol, non-containing wash syrup, b) dense glucose second crop solution, c) confectionery syrup and d) crystallic glucose. The carbohydrate composition of these solutions, determined by chromatography, is presented in Table 3. The isomerization process was conducted in solutions of substrates with 40% DS concentration, using the insoluble preparation

Table 6. Isomerization of glucose in a solution of hydrol purified with ion exchangers, DS = 68 (53% glucose content in DS) with the use of insoluble preparation Sweetzyme A, utilized in 14 batches of isomerization (temp. 65°C; pH = 7.0; concn. of hydrol solution — 40% DS; dose of enzyme in the first batch 10 GINU/g d. s. carbohydrate)

Process of isomerization		Absorbance (E) of 40% solution	Level of fructose produced (% in DS)	Degree of isomerization ID (%)
No. of batch	Time of isomerization (h)			
1	0	0.042	0	0
	24	0.410	22.0	41.5
2	0	0.048	0.85	1.6
	24	0.330	21.8	41.2
3	0	0.038	1.1	2.1
	24	0.279	21.5	40.6
4	0	0.038	0.95	1.8
	24	0.245	21.3	40.2
5	0	0.035	1.2	2.3
	24	0.220	20.9	39.4
6	0	0.038	0.90	1.7
	24	0.195	20.25	38.2
7	0	0.039	1.0	1.9
	24	0.175	19.35	36.5
8	0	0.038	1.1	2.1
	24	0.155	18.2	34.3
9	0	0.037	1.05	2.0
	24	0.140	17.2	32.5
10	0	0.041	1.25	2.4
	24	0.125	16.2	30.6
11	0	0.038	0.90	1.7
	24	0.112	15.05	28.6
12	0	0.040	0.95	1.8
	24	0.100	13.8	26.1
13	0	0.037	0.90	1.7
	24	0.090	12.3	23.2
14	0	0.036	0.85	1.6
	24	0.084	9.9	18.7

of glucose isomerase Sweetzyme A, applied many times in several consecutive batches of isomerization, after every single separation of the enzyme from the isomerized solution by sedimentation. Each isomerization batch was conducted at 65°C, pH adjusted to 7.0 for 24 h, with an addition of activators to the amount of 2 g $MgSO_4 \cdot 7 H_2O$ and 0.1 g

Table 7. Isomerization of glucose in a solution of confectionery syrup purified with ion exchangers, DE = 40.1 (21% glucose content in dry solids) with the use of insoluble preparation Sweetzyme A, utilized in 14 batches of isomerization (temp. 65°C, pH = 7.0; concn. of solution 40% in DS; dose of enzyme for the first batch of isomerization 10 GINU/g d. s. carbohydrate)

Process of isomerization		Absorbance (E), 40% solution	Level of fructose produced (% in DS)	Degree of isomerization ID (%)
No. of batch	Time of isomerization (h)			
1	0	0.020	0	0
	24	0.195	9.3	44.2
2	0	0.028	0.42	2.0
	24	0.160	9.2	43.9
3	0	0.030	0.44	2.1
	24	0.142	9.05	43.3
4	0	0.027	0.38	1.8
	24	0.128	9.0	42.8
5	0	0.025	0.34	1.6
	24	0.114	8.8	41.9
6	0	0.025	0.50	2.4
	24	0.105	8.5	40.6
7	0	0.026	0.48	2.3
	24	0.095	8.4	40.0
8	0	0.028	0.32	1.5
	24	0.088	8.2	39.0
9	0	0.025	0.46	2.2
	24	0.079	7.7	36.7
10	0	0.023	0.40	1.9
	24	0.070	7.25	34.5
11	0	0.022	0.36	1.7
	24	0.062	6 85	32.7
12	0	0.020	0.38	1.8
	24	0.057	6.4	30.4
13	0	0.020	0.42	2.0
	24	0.052	5.85	27.8
14	0	0.019	0.34	1.6
	24	0.048	5.25	24.9

$CoSO_4 \cdot 7\,H_2O/dm^3$ solution [7]. A fresh preparation of the insoluble enzyme Sweetzyme A was applied only in the first batch, in quantities corresponding to 10 GINU/g of glucose [7]. After decantation of the isomerized solution from the top of the sedimented enzyme, the enzyme was flooded with fresh preparation and the next batch of isomerization was

carried on. This procedure was repeated in 14 consecutive batches. The results of the studies on the process of isomerization carried out in this series of experiments are presented in Tables 4, 5, 6 and 7.

DISCUSSION

The experiments performed in the first part of the study showed that hydrolysates of potato starch with different contents of glucose in dry solids, from 21% in confectionery syrup to almost 100% in crystallic glucose, undergo the process of enzymatic isomerization to a satisfactory degree. The only exception was raw non-purified hydrol in which glucose was isomerized only in 32.5%. In all remaining starch hydrolysates, purified hydrol included, an over 40% conversion of glucose into fructose was obtained, and a certain quantity of reducing glucose elements of oligosaccharides was transformed into ketoses [1].

Isomerization was conducted in relatively concentrated solutions with 40% DS which has an essential meaning for industrial practice, due to reduced heat consumption during the final concentration of the products. Higher unitary results were obtained in case of the preparation of soluble glucose isomerase Optisweet P; it allowed to obtain a degree of isomerization higher than 50% in case of an almost pure glucose solution. In general, however, insoluble preparation of glucose isomerase such as e.g. Sweetzyme-A, is more suitable and, as shown by the results of the studies, it may be applied, depending on the substrate, 5-7 times, obtaining every time a degree of isomerization at the required level of about 40% or slightly lower.

Very good results are obtained with confectionery syrup with 40.1 DE as practically it is not inferior to pure glucose solution allowing for the same 7-fold utilization of enzyme. The effects of the isomerization process were somewhat lower in case of dense glucose solution for second crystallization which revealed the required degree of isomerization approx. 40% in six subsequent batches and in case of hydrol where a similarly high degree of isomerization was obtained in five batches.

It results from the experiments made that within the framework of each particular isomerization batch, the solutions of potato starch hydrolysates get brown as the process proceeds, however taking into consideration with the use of insoluble isomerase preparation, a weaker and weaker staining of solutions has been observed. On the average, the isomerized solutions of the 14th batch revealed an absorbance five times lower than in case of the first batch solutions.

It results from the experiments that enzymatic isomerization can be used to grade, i.e. significantly increase sweetness and to improve the taste of hydrol, a by-product of glucose and to better utilize the glucose solution intended for second crystallization because the latter process

would together with centrifugation and drying of the second product of glucose be then unnecessary. Isomerization of confectionery syrup, through an increase of its sweetness and a decrease of its crystalization abilities, allows to obtain a new type of syrup, especially suitable for fillings in the confectionery industry and in other food industries.

CONCLUSIONS

1. Enzymatic isomerization of glucose into fructose conducted with the use of soluble preparation of isomerase Optisweet P and insoluble preparation Sweetzyme A in various products of potato starch hydrolysis, gives good results allowing to obtain a degree of isomerization above 40%. The only exception is raw nonpurified hydrol which undergoes isomerization to a smaller degree (ID = 32.5%).

2. In case of repeated utilization of insoluble preparation of glucose isomerase, a satisfactory degree of glucose isomerization (above 40%) can be obtained, depending on the substrate, in 5-7 subsequent batches. Isomerization repeated in a number of batches higher than 7, gives results lower than 40%.

3. The enzymic isomerization of glucose in confectionery syrup, purified hydrol and second crop glucose solution increases significantly the sweetness and taste of these starch hydrolysates.

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PERIODYCZNA IZOMERYZACJA GLUKOZY W HYDROLIZATACH SKROBI ZA POMOCĄ ROZPUSZCZALNEGO I NIEROZPUSZCZALNEGO PREPARATU IZOMERAZY GLUKOZOWEJ

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Streszczenie

Półprodukty i produkty hydrolizy skrobi ziemniaczanej poddawane były procesowi periodycznej izomeryzacji enzymatycznej. Roztwory o stężeniu 40% s.s. hydrolu surowego o 75 DE, hydrolu oczyszczonego jonitami o 76 DE oraz hydrolu oczyszczonego i scukrzonego glukoamylazą o 83 DE poddano izomeryzacji w temp. 74°C, przy pH = 6,5 w czasie 24 godz. przy dawce rozpuszczalnego preparatu izomerazy glukozowej Optisweet — P w ilości odpowiadającej 20 TGIU/g glukozy w obecności soli magnezu i kobaltu jako aktywatorów. Uzyskano stopnie izomeryzacji glukozy (ID) w hydrolu surowym 32,5%, w hydrolu oczyszczonym 43,5%, w hydrolu scukrzonym glukoamylazą 50,0% oraz w celu porównania w roztworze glukozy krystalicznej o 99,9 DE — ID = 51,5%. W następnych doświadczeniach jako substraty służyły roztwory: a) hydrolu o stężeniu 40% s. substancji, oczyszczonego węglem aktywnym i jonitami, b) gęstego roztworu glukozowego do II rzutu krystalizacji o 84 DE, c) syropu cukierkowego o 40,1 DE i w celu porównania d) roztworu glukozy krystalicznej o 99,9 DE. Roztwory te były poddawane izomeryzacji w temp. 65°C, przy pH = 7,0 w obecności soli magnezu i kobaltu jako aktywatorów w ciągu 24 godz. Stosowano przy tym nierozpuszczalny preparat izomerazy glukozowej Sweetzyme — A w ilości odpowiadającej 10 GINU/g glukozy. Enzym dodawano tylko do pierwszej szarży izomeryzacji, a oddzielano z zizomeryzowanych roztworów przez sedymentację. Preparat izomerazy wykorzystywano do izomeryzacji świeżych roztworów substratów w 14 kolejnych szarżach.

Przeprowadzone badania wykazały, że dobre wyniki izomeryzacji glukozy do fruktozy, tj. stopień izomeryzacji ok. 40% lub powyżej uzyskano w przypadku roztworów glukozy krystalicznej i syropu cukierkowego w 7 kolejnych szarżach, a w przypadku roztworu glukozowego II rzutu w 6 szarżach, natomiast w przypadku roztworu hydrolu w 5 kolejnych szarżach izomeryzacji. Pomiary zabarwienia izomeryzowanych roztworów wykazały stopniowe ciemnienie (brunatnienie) roztworów w procesie izomeryzacji enzymatycznej w ramach każdej pojedynczej szarży. Natomiast porównanie zabarwienia zizomeryzowanych roztworów kolejnych szarż wykazało malejącą intensywność zabarwienia poczynając od pierwszej szarży izomeryzacji. Absorbancja roztworów zizomeryzowanych w czternastej szarży była około pięć razy mniejsza niż w pierwszej szarży.