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RECOVERY OF CRYSTALLINE GLUCOSE FROM THE LAST GREEN SYRUP (SO CALLED HYDROL)

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Key words: improvement of hydrol, glucose, glucoamylase ion exchanger.

Hydrol was refined and saccharified enzymatically, following which its glucose was crystallized directly or in a mixture with starch hydrolysate.

INTRODUCTION

Crystalline glucose is obtained from starch by acid-enzymatic hydrolysis or by two-stage exclusively enzymatic hydrolysis. Using the latter method, which involves the solubilization of starch with bacterial α-amylase and its subsequent saccharification with glucoamylase, it is possible to obtain reductivity in excess of 97 DE. However, this method requires two enzymatic preparations, and the introduction of a greater amount of enzymes impedes filtration of starch hydrolysates; the latter inconvenience, according to previous research, is mainly due to protein from the enzymatic preparations [7]. Hence many plants all over the world continue to produce glucose from starch by acid-enzymatic hydrolysis. This is done by dissolving the starch during preliminary hydrolysis with an acid (e.g. hydrochloric acid) up to reductivity of 16-22 DE, and then saccharifying the starch with glucoamylase up to the dextrose equivalent of 94-95 DE [2, 3]. After refining the hydrolysates with active carbon and concentrating them, the glucose is crystallized and separated in a centrifuge. The last green syrup after glucose crystallyzation known as hydrol, when undiluted with wash syrup (i.e. when the glucose of the second product was not washed with water in the centrifuge), has a concentration of about 60 Bx and a mean reductivity of 65 DE.

In addition to glucose, hydrol contains oligosaccharides due to incomplete saccharification of starch or to reversion. Certain non-carbohydrates

contained in starch also accumulate in hydrol. These include in particular the so called raw protein, mainly amino acids and peptides (ca. 1% in dry substance) and mineral substances (slightly over 2%) including sodium chloride (ca. 1.5%) formed during the neutralization of hydrochloric acid with soda. These impurities practically prevent further crystallization of glucose despite the fact that it constitutes half of the dry substance of hydrol [8].

Given the fact that the cost of glucose in hydrol used for, say, fodder is several times lower than the cost of extracted crystalline glucose, it was attempted in this research to recover from hydrol the greatest possible amount of glucose.

EXPERIMENTAL

METHODS

The following determinations were made in the course of experiments:

— concentration of solutions (Bx) with an Abbe refractometer (Zeiss),

— dry substance by drying solutions on absorption paper wads up to 105°C,

— reductivity (DE) by the Schoorl-Regenbogen method,

— absorbance of solutions in a Spekol photocolorimeter,

— content of raw protein total nitrogen according to Kjeldahl,

— mineral substances content by incineration at 600°C,

— quantitative carbohydrates content of various hydrol solutions by paper chromatography; 24 h chromatograms were developed by a 6 : 3 : 1 solution of n-propanol, water and ethyl acetate, developed with a reagent according to Buchan-Savage [1, 4]; the chromatographic spots were analysed with a reflectometer according to Sroczyński and Boruch [5, 6],

— amount of crystallized glucose $S = \frac{100(B_1 - B_2)}{100 - 1.1\,B_2}$, where B_1, is the concentration of the solution prior to crystallization (in % dry substance), and B_2 is the concentration of intercrystalline solution after crystallization (in % dry substance).

EXPERIMENTS

To carry out further crystallization of glucose remaining in the hydrol it was necessary to remove the mineral and organic impurities and to subject the refined hydrol to saccharification. The formation of new salts in the hydrol was prevented by abandoning the secondary acidic hydrolysis of oligosaccharides which would additionally lead to the appearance of unwanted products of glucose decomposition. The purified hydrol was

thus saccharified with glucoamylase. This method of enzymatic hydrolysis was advisable also because the refined hydrol could be combined with fresh starch hydrolysate after preliminary acid hydrolysis with ca. 22 DE reductivity, and the mixture then saccharified enzymatically. The hydrol dry substance-to-hydrolysate ratio was 1 : 4, which means that ca. 20% of hydrol formed as a by-product in the production of crystalline glucose was taken into account. The starch hydrolysate itself (22 DE reductivity) was also subjected to enzymatic saccharification for comparison. Given the viscosity and specific weight of hydrol, optimal refining with ion exchangers and adsorption resin could only be performed with a 1 : 1 water solution of hydrol with a concentration of 30-35 Bx which in fact corresponded to the initial concentration of the starch hydrolysate. The purification of the hydrol solution consisted in successively passing it (at 70°C) through a strongly acidic SD × 5 cation exchanger (from Xenon) with H^+ ions, a weakly basic Imac A21 anion exchanger with OH^- ions, and through Wofatit EA60 adsorption resin; in some cases the hydrol solution was additionally purified with Carbopol-0-extra active carbon. The refined hydrol solution was then saccharified at 60°C and pH = 4.5 for a lengthy period of time with Amyloglucosidase Novo II enzymatic preparation (manufactured by Novo Industri); the preparation addition amounted to 0.25% dry substance of the hydrol. Before and after saccharification the hydrol solution was sterilized for 10 min at boiling temperature. The saccharized hydrol solutions or 1 : 4 mixtures of hydrol and initial starch hydrolysate as well as pure hydrolysate of 22 DE reductivity serving as control were purified with 1% prepared silica (hyflocel), condensed after filtration to a ca. 72 Bx concentration, and then crystallized with 1.25% monohydrate glucose inoculate for several days in a temperature gradually lowered from 44 to 21°C. The obtained results are presented in Tables 1-4.

DISCUSSION

The experiments demonstrate that the purification of hydrol with cation exchanger, anion exchanger and adsorption resin (Tab. 1) leads to an almost 50-fold reduction of mineral substance content and a 30-fold reduction of raw protein content. The DE value is increased slightly from 64 to 68. Hydrol solution colour measured with absorbance changes from 0.16 to 0.02 and drops still further to 0.01 after treatment with active carbon. The saccharification of refined hydrol with glucoamylase increases reductivity to 84.6 after 72 h (Tab. 2) and to 86 DE after 96 h. In the mixture of refined hydrol and initial starch hydrolysate (1 : 4 proportion) the DE value after three days of glucoamylase treatment is 92.8. For comparison, the dense glucose juice of the second product in glucose-producing

Table 1. Refining of 1:1 solution of hydrol with ion exchanges and adsorption resin (temperature: 70°C, solution concentration: 33°Bx)

Solution	pH	Absorbance	Reductivity (DE)	Per cent content in dry substance of	
				mineral substances	raw protein
Unrefined hydrol	4.3	0.16	64.0	2.029	1.060
Hydrol after treatment with cation exchanger	0.7	0.09	66.5	0.292	0.798
Hydrol after treatment with anion exchanger	5.2	0.03	67.0	0.048	0.512
Hydrol after treatment with adsorption resin	5.4	0.02	68.0	0.047	0.033
Hydrol after treatment with active carbon	5.6	0.01	68.5		

plants displays reductivity of 85 DE, while the figure for glucose juice of the first product (after acid-enzyme hydrolysis) is 94.4 DE. During four days of saccharification of refined hydrol with glucoamylase (Tab. 3) there occurs a ca. 1.5-fold increase of glucose content, an almost 2-fold reduction of isomaltose and isomaltotriose content, and maltose with its higher homologues practically disappears. The glucose crystallization yield from refined and saccharified hydrol was 36.2% dry substance, and from the 1 : 4 hydrol-hydrolysate mixture it was 42.4%. This result must be considered rather good given the fact that the respective figure for glucose juice (pure hydrolysate) is 44.1% (Tab. 4).

Table 2. Enzymatic saccharification of 1:1 hydrol solutions before and after refining and of starch hydrolysate (glucoamylase Novo II addition amounting to 0.25% dry substance (temperature: 60°C, pH 4.5, solution concentration: 30°Bx)

Solution	Reductivity (DE) after saccharification-hours, for					
	0 h	24 h	48 h	72 h	96 h	120 h
Unrefined hydrol	64.0	64.4	64.9	65.5	66.2	65.5
Hydrol after treatment with cation a and anion exchangers and adsorption resin	68.0	77.5	82.5	84.6	86.0	82.8
Starch hydrolysate*	22.3	90.2	92.5	94.4	91.5	88.6
1:4 mixture of hydrol refined as above and starch hydrolysate*	31.5	89.6	91.5	92.8	92.0	89.0

*) after preliminary acid hydrolysis

Table 3. Changes of carbohydrates content in hydrol during refining and enzymatic saccharification with glucoamylase

Solution	Reductivity	Carbohydrates content in dry substance (%)						
		glucose	maltose	isomaltose	maltotriose	isomaltetriose	tetrasaccharides	pentoses and hexoses
Raw hydrol	64.0	48.6	5.1	25.3	7.6	7.6	2.5	traces
Hydrol refined with ion exchangers and adsorption resin	68.0	53.0	3.1	25.0	6.5	7.3	2.5	traces
Refined hydrol after 72 h of saccharification	84.6	72.1	traces	14.5	1.9	6.9	1.5	traces
Refined hydrol after 96 h of saccharification	86.0	75.8	traces	14.0	1	4.1	traces	0

[347]

Table 4. Crystallization of glucose monohydrate in solutions of glucose juice (from acid-enzyme hydrolysis), enzymatically saccharifiel 1:4 mixture of refined hydrol and initial starch hydrolysate, and in saccharified refined hydrol (inoculate: 1.125% crystalline glucise)

Crystallization time (h)	Temperature (°C)	Glucose juice (starch hydrolysate)			Saccharified 1:4 mixture of hydrol and starch hydrolysate			Saccharified hydrol		
		Bx	DE	per cent of crystallized glucose*)	Bx	DE	per cent of crystallized glucose*)	Bx	DE	per cent of crystallized glucose*)
0	44	71.9	94.4	0	71.9	92.8	0	71.8	84.6	0
24	36	64.1		28.8	69.5		11.9	69.7		10.0
48	29.5	59.2		39.0	62.1		33.3	67.6		18.8
66	24	56.1	77.6	44.1	58.8	77.5	40.0	65.2	75.4	25.3
72	23				58.4		40.8	63.6		30.2
90	21.5				57.5	75.8	42.4	60.8	71.8	36.2

*) glucose monohydrate converted to anhydrous glucose (in per cent of the initial solution)

CONCLUSIONS

1. Using ion exchangers and adsorption resin it is possible to remove nearly all mineral impurities and raw protein from hydrol.

2. Unlike raw hydrol which is only slightly saccharified with glucoamylase (a change from 64 to 66.2 DE), refined hydrol is saccharified to 86 DE, the reductivity value attained by glucose juice of the second product. The 1 : 4 mixture of refined hydrol and starch hydrolysate is saccharified by glucoamylase to 92.8 DE, a figure approaching that of saccharified pure hydrolysate (94.4 DE).

3. The carbohydrates content changes very little in hydrol during its refining, but changes are substantial during saccharification of refined hydrol with glucoamylase: glucose content increases by almost a half, the contents of isomaltose and isomaltotriose are reduced by about half, while the others oligosaccharides practically vanish altogether.

4. The amount of glucose crystallized from solutions saccharified with glucoamylase in the form of monohydrate, converted to anhydrous glucose was 36.2% in the case of refined hydrol, and 42.4% in the case of 1 : 4 hydrol-starch hydrolysate mixture; the latter figure is only slightly lower than that for pure glucose juice (44.1%).

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ODZYSKIWANIE KRYSTALICZNEJ GLUKOZY Z OSTATNIEGO ODCIEKU TZW. HYDROLU

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Streszczenie

W przeprowadzonych doświadczeniach, ostatni odciek po krystalizacji glukozy tzw. hydrol o wartości 64 DE, stanowiący produkt uboczny hydrolizy skrobi metodą

kwas-enzym poddawano rafinacji wymieniaczami jonowymi (kationitem i anionitem) oraz żywicą adsorpcyjną w celu usunięcia zanieczyszczeń mineralnych i organicznych (tab. 1), w tym głównie białka surowego. Następnie oczyszczony hydrol scukrzono glukoamylazą (0,25% s. s.) w temp. 60°C przy pH 4,5 w ciągu kilku dni (tab. 2). Jednocześnie scukrzano glukoamylazą hydrolizat skrobi o ok. 22 DE po wstępnej kwasowej hydrolizie i mieszaninę hydrolu rafinowanego i hydrolizatu w stosunku 1 : 4. Uzyskano znaczne scukrzenie hydrolu rafinowanego do wartości 86 DE, równającej się redukcyjności soku glukozowego produktu II. Scukrzanie glukoamylazą mieszaniny hydrolu i hydrolizatu pozwoliło osiągnąć redukcyjność 92,8 DE zbliżoną do wartości 94,4 DE soku glukozowego produktu I (samego hydrolizatu).

Przeprowadzona analiza składu węglowodanowego hydrolu surowego i hydrolu rafinowanego po jego scukrzeniu glukoamylazą pozwoliła stwierdzić podwyższenie prawie 1,5-krotne zawartości glukozy i obniżenie do około połowy zawartości izomaltozy i izomaltotriozy w s. s. hydrolu oraz praktycznie zanik pozostałych oligosacharydów rj. maltozy i jej wyższych homologów.

Po zagęszczeniu wymienionych roztworów, scukrzonych glukoamylazą, do ok. 72 Bx i zaszczepieniu ich 1,25% monohydratu glukozy krystalicznej, przeprowadzono w ciągu 2-4 dni w temperaturze stopniowo obniżanej z 44°C krystalizację w nich glukozy. W wyniku tego procesu uzyskano 36,2% glukozy krystalicznej (w przeliczeniu na bezwodną) z hydrolu rafinowanego scukrzonego a nawet 42,4% wykrystalizowanej glukozy z mieszaniny hydrolu i hydrolizatu skrobi (1 : 4) to jest ilość zbliżoną do glukozy wykrystalizowanej z czystego hydrolizatu skrobi — 44,1%, co jest dobrym rezultatem. Powyższe wyniki mają istotne znaczenie z uwagi na kilkakrotnie wyższą cenę glukozy krystalicznej niż glukozy zawartej w hydrolu.