A C T A A L I M E N T A R I A P O L O N I C A
Vol. VI (XXX), No. 1-2 1980

MIECZYSŁAW BORUCH
EWA NEBESNY

EFFECT OF GLUCOSE ISOMERASE ON OLIGOSACCHARIDES IN PRODUCTS OF STARCH HYDROLYSIS

Department of Chemical Food Technology, Technical University, Łódź

Key words: starch, glucose isomerase, maltose, glucose ketose, isomerization.

Solutions of hydrolysates of starch and maltose were treated with glucose isomerase. Chromatographic analyses of the products of isomerization indicated glucose isomerase transforms not only free glucose into fructose but also some minor quantity of oligosaccharides into appropriate sugars containing ketose.

Glucose isomerase (ketol-isomerase of D-glucose) is an enzyme showing capability for transforming glucose into fructose. In the process of enzymatic isomerization between 40 and 54% of glucose can be transformed into fructose, depending on conditions in which such a reaction is carried out [4, 6]. According to the results of Geyer [3] and of other authors, glucose isomerase specifically affects glucose in starch hydrolysates unchanged: maltose, maltotriose and higher oligosaccharides of the homologic chain of maltose. The isomerization consists in transformation of aldose in its aldehyde form, D-glucose, into ketose i.e., D-fructose.

The ketose resulting from isomerization can be clearly observed and determined quantitatively by means of chromatographic analysis performed with a resorcine developer. Under these circumstances the chromatographic stain of ketose acquires a pink-red colour through resorcine [5]. Effects of isomerization of glucose into fructose can also be determined with the polarimetric method. The process of isomerization of glucose in starch hydrolysates can be performed periodically by means of soluble preparation of isomerase for a sinle use, or with a nonsoluble one for multiple use. It can also be done in a continuous way with a preparation of immobilized glucose isomerase [2, 3, 7].

The paper aims at proving that glucose isomerase is capable not only of transforming free glucose into fructose but that under special conditions it also transforms minor quantities of glucose reducing elements in molecules of oligosaccharides. This was carried out by glucose isomerase under optimal conditions acting on solutions of maltose and starch hydrolysates free of glucose or with low levels of it. Products of isomerization were analysed chromatographically to determine the content of ketoses.

MATERIALS AND METHODS

The study involved analysis of continuous isomerization of glucose in starch hydrolysates with different degrees of saccharification, e.g., in a solution of pure glucose (DE = 100), in a starch hydrolysate solution D = = 45, and in solutions of pure maltose and starch hydrolysate with DE = = 31 from which glucose was completely separated by binding it in a strongly basic anionite Wofatit SBW with borate ions [1]. The continuous enzymatic isomerisation was applied to solutions of these starch hydrolysates with 40% dry substance. Calcium ions were released by demineralization with ion exchangers. The solutions were then supplemented with magnesium sulphate as activator up to concentration of 4 m mole Mg^{++}. Solutions of substrates with pH = 8.5 were passed through a bed of glucose isomerase (Sweetzyme S) placed in a column with thermostat at 65°C [9] and the speed of 1 volume of solution per hour in terms of the volume of the enzyme bed.

Progress of the enzymatic isomerization and the obtained degree of isomerization (ID) were measured polarimetrically, determining the angle of turning of the plane of light polarization by using a Hilger-Watts polarimeter. Solutions of substrates before isomerisation as well as the solutions after enzymatic isomerisation were placed on chromatographic paper Whatman 1 in quantities corresponding to 250, 500 and 1000 μg of dry substance, and in the case of maltose alone — 2000, 3500, 5000 μg of dry substance.

For the purpose of quantitative assessment of sugars in the chromatographic stains also standard solutions containing varying quantities (15 to 250 μg dry substance) of glucose, maltose, isomaltose, maltotriose and fructose.

The chromatograms were developed with the flow method over 48 hrs with a solution composed of n-propanol: water: ethyl acetate (6:3:1).

One set of chromatograms from each of the analyzed solutions was developed after Buchan-Savage with the following solution: 4g dipheny-

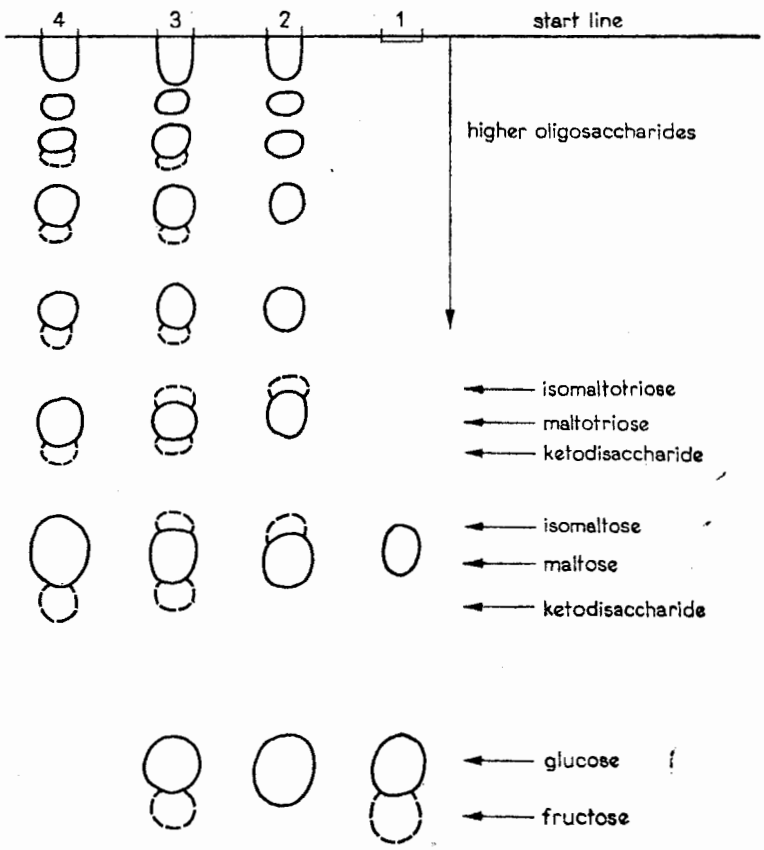

Fig. 1. Reproduction of the chromatogram developed with the Buchan-Savage reagent; 1 — standard solution of fructose, glucose and maltose; 2 — starch hydrolysate before isomerization; 3 — starch hydrolysate after isomerization; 4 — isomerized starch hydrolysate after release of glucose from it

Table 1. Enzymatic isomerization of maltose into ketodisaccharide

Quantity of applied maltose μg	Quantity of formed ketodisaccharide μg	%
2000	35	1.75
3500	50	1.43
5000	75	1.50

lamine, 4 ml aniline, 20 ml 85% orthophosphoric acid supplemented with acetone to 200 ml. Drying time: 10 min, temperature — 80°C [8]. Another set was developed in 0.1 g rezorcine in 200 ml acidified ethanol. Time: 10 min., temp. 70°C [4].

RESULTS

The chromatograms developed after Buchan-Savage showed the following stains in the case of a solution of starch hydrolysate before isomerization: glucose — olive/green, maltose — blue, isomaltose — olive/green, and higher oligosaccharides — blue. Near the start line there was a blue band of dextrins.

In the case of solution of starch hydrolysates subjected to isomerization the chromatogram developed after Buchman-Savage showed in addition to before glucose a rusty (brown-red) stain of fructose and before maltose (which was poorly marked) a violet stain of an unidentified disaccharide.

Similar violet stains with very poor colouring, an evidence of trace quantities of unidentified oligosaccharides, were observed in spots preceding blue spots of maltotriose and higher oligosaccharides in the homologic chain of maltose.

The chromatograms developed with rezorcine reagent showed after isomerization pink-red stains in positions corresponding to unidentified oligosaccharides, mostly before maltose (stain) and less intensive stains before maltotriose, maltotetrose as well as traces of a pink-coloured oligosaccharide before maltopentose. Similar, only more intensively coloured stains of newly developed oligosaccharides were observed in chromatograms of isomerized solution of starch hydrolysate, which was freed of glucose before isomerization. Chromatograms of the isomerized solution of maltose showed immediately before the maltose stain a very distinct unidentified disaccharide. Its colour was violet when developed after Buchan-Savage and pink-red after the rezorcine (Fig. 2).

Comparing the pink stains formed during isomerization of oligosaccharides with the pink stains of the standard solutions of fructose, the authors were able to approximate the content of ketose in the stains of the disaccharide so far unidentified and formed in effect of isomerization of the solution of pure maltose.

Since the transormation concerned maltose containing two glucose, elements, and isomerization may take place only on one glucose element which undergoes tautometry, therefore the transformation of aldose into ketose occured only in one half of the molecule of maltose. Consequently, when 1.5% ketose formed from maltose is observed in a chromatographic stain of the ketodisaccharide developed during isomerization, we can say that there a double amount of the latter as compared to ketose, that is, 3% in reference of the initial volume of maltose.

The experiments showed that glucose isomeraze used in isomerization of glucose affects glucose primarily by changing it into fructose but when there is a low level of glucose substrate in the isomerized solution, and even more so when glucose is absent, the glucose isomerase acts very

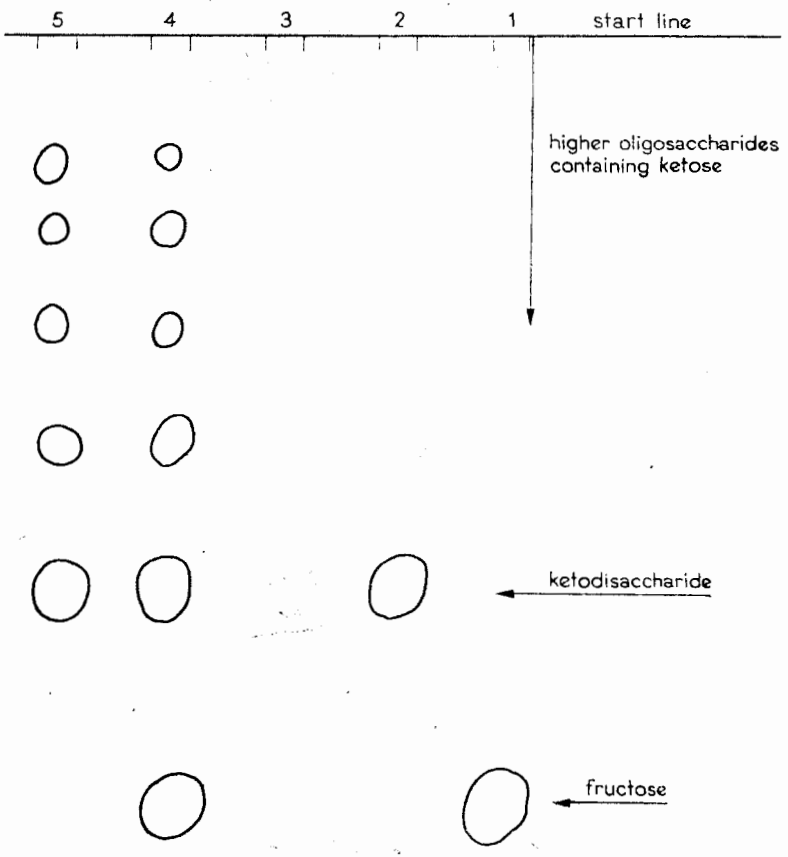

Fig. 2. Reprodution of the chromatogram developed with the rezocine reagent; 1 — standard solution of fructose; 2 — isomerized solution of maltose; 3 — starch hydrolysate before isomerization (negative reaction); 4 — starch hydrolysate after isomerization; 5 — starch hydrolysate without glucose after isomerization

slowly also on oligosaccharides containing the glucose element that undergoes tautometry, and on maltose in particular (Table 2).

The degree of isomerization defined in Table 2 (ID) does not embrace higher oligosaccharides containing the fructose element which are formed during isomerization.

CONCLUSIONS

Although full identification of the disaccharide formed from maltose during isomerization was not carried out on account of lack of a proper standard of the so far unknown ketodisaccharide yet analyses with rezorcine on chromatograms as well as lowering of specific rotation

Table 2. Isomerization of starch hydrolysates by means of immobilized glucose isomerase Sweetzyme S (substrates concentration — 40% d.s.)

Substrate of isomerization % d.s.	Contents of glucose before isomerization % d.s.	Contents of maltose before isomerization % d.s.	Contents of fructose after isomerization % d.s.	Contents of ketodisaccharide after isomerization % d.s.	Degree of isomerization of glucose rest ID
Glucose	100	0	47.5	0	47.5
Starch hydrolysate 45 DE	25	22.3	11.8	1	47.2
Starch hydrolysate 31 DE free of glucose	0	33.6	0	2	6.0
Maltose	0	100	0	3	3

after isomerization prove unmistakably that the unidentified carbohydrates contain an element of ketose in their melaculose.

Ketose rests were found not only in the chromatographic stains representing disaccharides after isomerization but also in the stains from higher oligosaccharides (as stains preceding the latter).

In every case it can be said that glucose isomerase acts upon not only glucose, as it has been maintained so fat, but also on oligosaccharide (although not very intensively) which appear together with glucose in products of starch hydrolysis, The effect is stronger the less glucose in the solution.

Partial isomerization of oligosaccharides in starch hydrolysate solutions can be one of the reasons for lower productivity of fructose as compared with an analogical process of treting a pure glucose substrate with glucose isomerase.

The above experimentation is continued by the authors and it is aimed at precise identification of isomerized oligosaccharides as well characterization of their properties.

LITERATURE

1. Boruch M.: Zeszyty Naukowe PŁ, Chemia Spożywcza 1968, 14, 5.
2. Grams Th. C.: Stärke 1976, **28**, 344.
3. Geyer H. U.: Stärke 1974, **26**, 225.
4. Novo Ind. — Novo Enzyme Information 1975, 089c-e.
5. Opieńska-Blauth J. at al: Chromatografia, PWN, Warszawa 1957, 594, 597.
6. Schnyder B. J.: Stärke 1974, **26**, 12, 409.
7. Schwengers D., Schneider H. G.: DOS 2.402.226 an Pfeifer und Langen Köln.
8. Schwimmer S., Bevenue A.: Science 1956, 3, 543.
9. Zittan L., Poulsen P. B., Hemmingsen St. H.: Stärke 1975, **27**, 236.

Manuscript received: January, 1979
Authors address: 90-537 Łódź, Gdańska 166

M. Boruch, E. Nebesny

DZIAŁANIE IZOMERAZY GLUKOZOWEJ NA OLIGOSACHARYDY W PRODUKTACH HYDROLIZY SKROBI

Instytut Chemicznej Technologii Żywności, Politechnika, Łódź

Streszczenie

Podczas prowadzenia badań procesu enzymatycznej izomeryzacji glukozy w hydrolizatach skrobi zauważono, że w szczególnych warunkach izomeraza glukozowa działa nie tylko na glukozę, lecz również na oligosacharydy towarzyszące glukozie

w hydrolizatach. Obserwacje te polegały na stwierdzeniu na chomatogramach bibułowych roztworów hydrolizatów poddanych długotrwałej izomeryzacji, plam odpowiadających fruktozie oraz oligosacharydom zawierającym w swoich cząsteczkach element fruktozowy (plamy wywołane rezorcyną). W następstwie tego spotrzeżenia wykonano szczegółowe badania poddając długotrwałemu działaniu izomerazy glukozowej roztwory czystej maltozy i roztwory hydrolizatów skrobiowych pozbawionych całkowicie glukozy przez związanie jej na anionicie w postaci kompleksu boranowego. Wyniki analizy chromatograficznej produktów izomeryzacji takich roztworów wykazały, że maltoza oraz wyższe oligosacharydy w próbach pozbawionych glukozy ulegają częściowo izomeryzacji, tworząc odpowiednie oligosacharydy zawierające w swych cząsteczkach po jednym elemencie fruktozowym. Stwierdzono na przykład, że czysta maltoza poddana działaniu izomerazy glukozowej ulega w ok. 3% przemianie w odpowiedni ketodwucukier. Podobnie przedstawia się wynik izomeryzacji oligosacharydów w zmodyfikowanym hydrolizacie skrobi z tym, że obok 2% ketodwucukru pozostają jeszcze nie oznaczone ilości wyższych ketooligosacharydów.