STUDIES ON SUGARS IN ACID AND ENZYME HYDROLYSATES OF POLYSACCARIDES III. ON THE SUGAR COMPOSITION OF THE COMMERCIAL GLUCOSES PRODUCED BY ACID OR BY ENZYME HYDROLYSIS FROM SWEET POTATO STARCH

STUDIES ON SUGARS IN ACID AND ENZYME HYDROLYSATES OF POLYSACCARIDES

III. ON THE SUGAR COMPOSITION OF THE COMMERCIAL GLUCOSES PRODUCED BY ACID OR BY ENZYME HYDROLYSIS FROM SWEET POTATO STARCH*

By

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In a previous paper of this series, the sugar composition of hydrol of sweet potato starch which was hydrolyzed by oxalic acid (Part 1) and the sugar composition in the concentrated H_2SO_4 hydrolysates of wood (Part 2) were reported (1, 2). We now report on the sugar composition of commercial glucoses produced by mold enzyme preparation from sweet potato starch. In Japan the industrial production of glucose from sweet potato starch by mold enzyme preparation was established in these years. The total and reducing sugar contents of nine commercial glucoses (three powdered glucoses produced by mold enzyme preparation, three powdered and three crystalline glucoses produced by oxalic acid hydrolysis of sweet potato starch) were analyzed, and also paper chromatography and separative determination of sugars of the samples were carried out.

Tokuoka (1941) suggested that Aspergillus oryzae produced glucosidase with the ability to form glucose directly from starch (3). Corman (1948) reported that some mold enzyme produce glucose directly from starch (4). Phillips (1951) isolated gluc-amylase from Rhizopus delemar and studied on the purification and its properties (5). Tsujisaka, Hukumoto and Yamamoto (1958) have succeeded to obtain in a crystalline state the two saccharogenic amylases of Rhizopus delemar and Aspergillus niger (6). The two crystalline

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amylases formed glucose directly from starch. Komaki succeeded to liquify a sweet potato starch by commercial α-amylase and produce glucose directly by commercial gluc-amylase in the industrial plant (7). During the past few years, the industrial production of glucose by these enzyme from sweet potato starch has been rapidly developing in Japan. It is reported that it is now producing 150 metric tons per day and will be almost 700 metric tons at the end of 1960 (8). The outline of the manufacturing method of a commercial glucose in Japan is as follows:

(A) Acid hydrolysis

Sweet potato starch ⟶ Saccharification by oxalic acid ⟶ Neutralization by lime

⟶ Purification by ion exchange resin and activated carbon ⟶ Concentration ⟶ Powdered glucose

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Crystallization ⟶ Crystalline glucose

(B) Enzyme hydrolysis

Sweet potato starch ⟶ Liquifaction by bacterial amylase ⟶ Saccharification by amylase of Rhizopus delemar or Asp. niger

⟶ Purification by ion exchange resin and activated carbon ⟶ Concentration ⟶ Powdered glucose

↓

Crystallization ⟶ Crystalline glucose

There was a strong and also a weak point in the method of hydrolysis by acid and by enzyme preparation. By acid hydrolysis, the cheap cost of acid; short time for hydrolysis; almost 90 per cent of hydrolysis limit; reversion products, especially bitter taste of gentiobiose and a large amount of hydrol can not be neglected. On the other hand, by enzyme hydrolysis it takes long hours and numerous vesseles for hydrolysis, and enzyme preparations are rather expensive. But initial concentration (40~50%) of starch for hydrolysis is higher than the former and the limit of hydrolysis is over 98%. And so, it crystallizes very easily. Especially the reversion products, for example bitter taste gentiobiose, are not produced. From these facts, in some case the separation of the mother liquor is not necessary for definite food uses.

The use of commercial glucose as well as the selection and development of methods for its analytical evaluation are dependent on adequate knowledge of its composition. The objective of the present work, therefore, was to analyze the commercial glucoses in enough detail to obtain concrete evidence of both the amounts and the type of sugars in them. The work was developed as a fundamental exploration including three phases: (a) use of a direct method for the determination of the total and reducing sugar content of commercial glucose, (b) qualitative analysis of sugar component by paper chromatography, (c) quantitative analysis of sugar component.

All samples were furnished at the end of 1959 by the manufacturing incorporation through the Institute of Food Research, Department of Agriculture and Forestry.

Experimental

Nine samples of commercial glucoses were used in these studies. Sample of Nos. 1~3 are produced by enzyme hydrolysis; Nos. 4~6 by acid hydrolysis; Nos. 7~9 are centrifuged crystalline samples produced by acid hydrolysis.

(1) Determination of Total and Reducing Sugar.

The total and reducing sugar contents were determined by the Bertrand-Henmi method due to the Tokyo University standard procedures. The obtained results are shown in the first and second lines of Table 1.

The contents of total sugar were not considerably different in all samples, but the contents of reducing sugar in the commercial glucoses produced by enzyme preparation were in larger amount than that of powdered glucoses produced by acid hydrolysis. As it is a commercial product, in crystalline glucoses, there are small amounts of dextrin (truly, they are isomaltose, gentiobiose and etc.) (Nos. 7, 8, 9).

(2) Identification of Sugar Component by Paper Chromatography.

(A) Oligosaccharides and levoglucosan

Twenty per cent solution of each sample was made. Aliquats of 5~6 μl of the solution were applied to a Toyo No. 2 filter paper sheet. Pyridine, butanol and water (4:6:3) was the solvent used for all ascending chromatograms. The spots were made visible with silver nitrate and aniline hydrogen phthalate. The obtained chromatogram is shown in Fig. 1.

(i) *Powdered glucoses produced by enzyme preparation*

From the Table 1, there is no considerable difference in the value of total and reducing sugar, but sugars other than glucose in the enzyme hydrolysis samples are smaller than that of the acid hydrolyzed samples and there are two types. The one contain a small amount of isomaltose which is derived from the branched point of amylopectin (No. 1 and 3). The other contains a larger amount of maltose than isomaltose (No. 2), and also 1,4-α-linked oligosaccharides which were identified as panose, maltotetraose and maltopentaose from their R_f value. It is a very interesting fact concerning the earlier report of Whelan on the hydrolysis mechanism of starch by mold enzyme (7) that the hydrolyzates contained a larger amount of maltose than that of maltotriose and a larger amount of maltotetraose and maltopentaose. At any rate, there are few amounts of oligosaccharides and further detail will be reported later after isolation by carbon column chromatography.

(ii) *Powdered and crystalline glucoses produced by acid*

Table 1. Sugar composition of commercial glucoses.

	Enzyme Glucoses				Acid glucoses					
					Powder			Crystalline		
Sample No.	1	2	3		4	5	6	7	8	9+
Total sugar	91.11	88.20	93.46	Total sugar	89.76	88.75	90.68	91.30	91.68	89.64
Reducing sugar	88.48	85.35	92.32	Reducing sugar	81.06	82.34	81.15	90.78	90.99	88.24
Dextrin	2.37	2.57	1.03	Dextrin	7.83	5.77	8.57	0.47	0.62	1.26
Glucose (+Fructose)	87.45	82.01	89.29	Glucose	77.47	78.14	76.33	91.09	91.14	85.63
Maltose etc.	0.71	2.61	1.27	Maltose etc.*	4.42	2.60	5.37	—	—	1.21
Isomaltose etc.	1.94	0.26	2.30	Isomaltose, Gentiobiose etc.	5.74	6.64	5.93	—	—	2.40
Maltotetraose etc.	0.34	0.54	0.11	Panose etc.	1.07	0.71	1.69	—	—	0.09
Maltopentaose etc.	0.37	0.55	0.22	Isomaltotriose etc.	0.65	0.41	0.61	—	—	0.13
Higher Oligosaccharides	0.29	2.23	0.93	Higher Oligosaccharides	0.43	0.27	0.75	—	—	0.16
Total Oligosaccharides	3.66	6.19	4.17	Total Oligosaccharides	12.29	10.61	14.35	0.21	0.53	4.01

* Besides the Maltose, it's containing Kojibiose, Sophorose, Nigerose, Laminaribiose, Cellobiose etc. produced by acid reversion.

\+ Under the regular grades.

The difference between the value of total and reducing sugars is larger than that of the samples produced by enzyme preparation. So there are larger amounts of oligosaccharides. In paper chromatograms of sample Nos. 4~6,

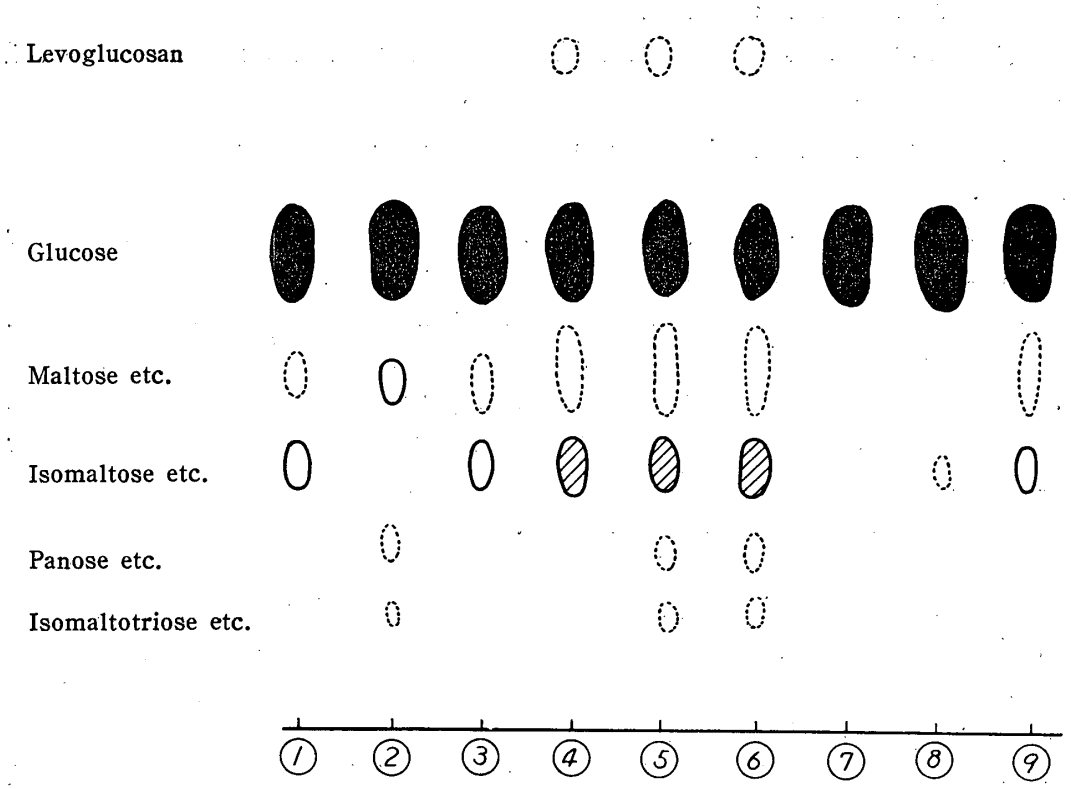

Fig. 1. Paper chromatogram of commercial glucoses.

the spot of maltose, the continued spots of kojibiose, sophorose, nigerose, laminaribiose, cellobiose and etc. produced as the reversion products were recognized. Also the spots of levoglucosan and hydroxymethyl furfural were identified. These spots are characteristic for the acid hydrolysates of starch.

In the paper chromatogram of crystalline sample (Nos. 7~9) faint spots of oligosaccharides were recognized. From this results, the spots of isomaltose, gentiobiose and other reversion sugars were identified just the same as the powdered glucoses.

(B) Fructose

When a paper sheet was developed with pyridine, butanol and water (4:6:3) using the descending method a small spot which has higher R_f value than that of glucose was recognized in the same samples, using resorcin instead of AHP as the coloring reagent. This R_f value was the same as arabinose, mannose and fructose. When the spots are made visible with resorcin, it colored reddish pink (ketose reaction) and on the other hand, when a paper sheet was developed with phenol, butanol, acetic acid and water (20:20:8:40) using the 2~3 times multiple descending method, these spots of

large spots of isomaltose and gentiobiose were recognized. Further, except arabinose, mannose and fructose are perfectly separated. Thus, this spot of sugar is identified as fructose by a paper chromatographic comparison of its R_f value with authentic sample.

By these methods fructose was identified only in the sample of No. 1 and No. 2 which are hydrolyzed by enzyme.

(3) Determination of Sugar Component by Paper Chromatographic Method.

Aliquots of 0.1 *ml* of 20% solution were applied to a Toyo No. 2 filter paper sheet (20×40 cm) along a line previously drawn parallel to the bottom of the sheet. The same solvent was used for two times multiple development. Drying the resulting chromatogram. Cut into strips along the longtitudinal lines and develop the locator strips with silver nitrate reagent. Locate the respective sugars and excise appropriate sections of the chromatogram. After extraction of sugar from each section, the determination was made by the somogyi reagent as is shown in Table 1.

The average value of total sugar of the samples produced by enzyme preparation was 90.91% and that of samples produced by acid hydrolysis was 89.73%. Thus, there was no considerable difference. But it is worthy of notice that the average content of true glucose of the former was 86.73% and the latter was 77.31% and there was considerable difference. Also the average content of oligosaccharides of the former was 4.67% and the later was 12.4%. The crystalline samples contained very small amounts of oligosaccharides (No. 7 was 0.21% and No. 8 was 0.53% respectively) except for sample No. 9 (4.17%).

(4) Determination of Fructose in the Glucoses produced by Enzyme Preparation.

As above described, the presence of fructose was recognized. Then the determination of fructose in the samples produced by enzyme preparation was made. One new sample (No. 10) was added which was produced by enzyme preparation. The separative determination of glucose with fructose by paper chromatographic technique was very difficult. Because the R_f value of glucose was not so different from that of fructose on the chromatogram developed with pyridine, butanol and water (4:6:3) and there were large amounts of glucose and very small amounts of fructose.

Solvent selection was based on the rate of movement of fructose and efficiency of separation of glucose. Thus phenol, butanol, acetic acid and water (20:20:8:40) was the solvent used for three times multiple ascending chromatograms (the R_f value of fructose is 0.51 and that of glucose is 0.37). Cut into strips along the longtitudinal lines and develop the locator strips

with silver nitrate reagent. Locate the respective sugars and excise appropriate sections of the chromatogram. After extraction of sugar from each sections, the determination was made by the Somogyi reagent as is shown in Table 2 (Oligosaccharides were analyzed after extracting with hot water and hydrolyzing by acid).

Table 2. Per cent of fructose, glucose and oligosaccharides in the commercial glucoses produced by enzyme preparation.

Sample No.	Fructose*	Glucose	Oligosaccharides*
1	0.86	95.08	4.06
2	1.94	91.37	6.69
10	1.08	88.88	10.04

* The values were calculated as glucose.

Since the analytical values of fructose in Table 2 were calculated as glucose, the standard curve of fructose was made by the Somogyi reagent [1/200N, $Na_2S_2O_3 \cdot 5H_2O$ (F=1.034), 1 *ml*=0.1420 mg of fructose=0.1402 mg of glucose]. From this results, the contents of fructose are shown in Table 3.

Table 3. The contents of fructose in the commercial glucoses produced by enzyme preparation.

Sample No.	Fructose	Per cent in total sugar
1	0.79	0.87
2	1.73	1.79
10	0.98	1.09

The content of fructose in the commercial glucoses produced by enzyme preparation accounted for about 0.8~1.7%. The mechanism of fructose formation will be reported elsewhere.

Discussion

As the number of samples is too small to discuss the general component of sugars in the commercial glucoses, we will continue these determinations. From the above results, it is obvious that the two type of commercial glucoses produced by acid and by enzyme preparation showed almost the same content of total sugar, but the former showed a smaller amount of reducing sugar and true glucose and a larger amount of oligosaccharides including reversion products than the latter. From these points, the manufacturing production of glucose by enzyme preparation give promise for future development.

From the view of sugar component, enzyme preparation hydrolyzed starch almost 100% might be made from Rhizopus culture while the enzyme prepa-

ration which hydrolyzed starch almost 70~80% containing not only saccharogenic but transglycosylic activity will be produced from Aspergillus niger. The latter give also promise for future development for another purpose.

About 1% of fructose was recognized to be present in the commercial glucoses produced by enzyme preparation and it seems to effect a browning reaction and absorption of moisture. The reason for fructose formation seems to be as follows: (1) there are fructose-linkage in raw starch of sweet potato, (2) crude enzyme preparation contain fructose derived from mold-bran, (3) enzyme preparation has isomerase activity, (4) isomerization by ion exchange resin which is used for purification.

But on the first problem we have no report on the fructose linkage in the starch. On the second problem fructose could not be recognized in the enzyme preparation by our examination. On the third problem, fructose isomerase may be a future problem. On the fourth problem, many technologists of manufacturing plants inform us that after purification of starch hydrolysate by ion exchange resin a fructose spot was recognized sometimes by paper chromatography in spite of no presence before. Phillips and Pollard reported on the decomposition of sugars by anion exchange resin (10). Rebenfeld and Pacsu, Turton and Pacsu, and Buhler wrote on the isomerization of glucose to fructose by anion exchange resin (11, 12, 13). Also, Langlois obtained a patent which produced fructose applying strong basic exchange resin on glucose solution or corn syrup (14). We will examine this problem in the near future.

Acid hydrolysis has a good point; the time for hydrolysis is short and acid is cheaper than enzyme preparation. But the reversion products, namely, the large amounts of hydrol can not be neglected. The content of oligosaccharides which is shown in Table 1 is unavoidable in manufacturing production. In America, hydrol is said to be of considerable commercial importance. It is used chiefly as an ensilage supplement and in stock feeds. But in Japan it is expensive and its effective utilization is not yet established.

Isomaltose and panose which are contained in the glucoses produced by enzyme preparation seems to be derived from the branched point of amylopectin (waxy maize starch), employing a frequency ratio of 24:1 and a relative hydrolysistic rate of 1:4 for the maltose : isomaltose disaccharides linkage, showed that the maximum concentration of isomaltose would occur at 91% hydrolysis where 3.4% isomaltose (42% of the original 8%) could be expected (15). In the sweet potato starch, amylose is about 80% and the average chain length is 27. Then the calculated results for 1:6-α-branched point isomaltose is about 6%. From our determination the isomaltose content of the commercial glucoses produced by enzyme preparation is 0.3~2.3% and therefore enzyme preparation would cleavage the 1, 6-α-linkage of amylopectin.

The presence of a small amount of nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) was shown in the oligosaccharides fraction of the glucoses produced by enzyme preparation isolated by carbon column chromatography. Wolfrom et al. showed definite evidence for the presence of 1, 3-α-linkage (nigerose) in the waxy maize starch (16). This evidence consists of the isolation of nigerose as its crystalline β-D-octaacetate from waxy maize starch acid hydrolyzate produced under a condition in which the formation of this disaccharide during the hydrolysis is negligible. Therefore, the small amount of nigerose will remain in them because of no cleavage by the enzyme.

Summary

The total and reducing sugar contents of nine commercial glucoses (three powdered glucoses produced by enzyme preparation, three powdered and three crystalline glucoses produced by acid hydrolysis from sweet potato starch) were analyzed, and paper chromatography and separative determination of sugars of the samples were carried out.

(1) The contents of total sugar were not considerably different in all samples, but the contents of reducing sugar in the commercial glucoses produced by enzyme preparation were of larger amount than that of powdered glucoses produced by acid hydrolysis.

(2) The average content of true glucose in the commercial glucoses produced by enzyme preparation was 86.3%, which was larger in amount than that of powdered glucoses produced by acid hydrolysis (77.3%). The average content of oligosaccharides of the commercial glucoses produced by enzyme preparation was 4.2%, which was less than that of glucoses produced by acid hydrolysis (12.4%).

(3) By paper chromatography, levoglucosan was detected in the commercial powdered glucoses produced by acid hydrolysis, while the glucoses produced by enzyme preparation did not contain levoglucosan. Fructose was detected in some commercial glucoses produced by enzyme preparation, and its content was about 1% of the total sugar.

(4) The main oligosaccharides of the commercial powdered glucoses produced by acid hydrolysis were isomaltose and gentiobiose; and also, kojibiose, sophorose, nigerose, laminaribiose, maltose, cellobiose produced by acid reversion of glucose were detected. The crystalline glucoses produced by acid hydrolysis contained a very small amount of the reversion products (0.37%).

(5) There were two types of the commercial glucoses produced by enzyme preparation, one of which contained a larger amount of isomaltose than that of maltose, and the other contained a larger amount of maltose than that of isomaltose.

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