# STUDIES ON SUGARS IN ACID AND ENZYME HYDROLYSATES OF POLYSACCHARIDES IV. ON THE β-LINKED SUGARS IN THE OLIGOSACCHARIDES FRACTION OF THE COMMERCIAL GLUCOSES PRODUCED BY ACID OR BY ENZYME HYDROLYSIS FROM SWEET POTATO STARCH

# STUDIES ON SUGARS IN ACID AND ENZYME HYDROLYSATES OF POLYSACCHARIDES

## IV. ON THE β-LINKED SUGARS IN THE OLIGOSACCHARIDES FRACTION OF THE COMMERCIAL GLUCOSES PRODUCED BY ACID OR BY ENZYME HYDROLYSIS FROM SWEET POTATO STARCH*

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In a previous paper (1) of this series, the sugar composition of the commercial glucoses produced by acid or enzyme preparation was quantitatively determined by the paper chromatographic method. Isomaltose and gentiobiose were identified in the samples but two spots of these sugars overlapped on a paper chromatogram and were, therefore, determined as their total value. There were many kinds of oligosaccharides reversed from glucose—mainly they were gluco-bioses—in the acid hydrolysate of starch. Experimental evidences of earlier workers on the condensation of glucose in the presence of acid with the formation of glucosidic linkage point to yielding not only α-linkage but also β-linkage and demonstrate that the 1, 6-linked disaccharides, isomaltose and gentiobiose, are dominant.

On the other hand, there were α-linked maltose, isomaltose and panose which would seem to be the partial hydrolysates of amylopectin and amylose of raw starch in the samples hydrolyzed by enzyme preparation made from *Rhizopus* culture and would not contain transglucosylase activity.

For the comparison of two methods of hydrolysis or the classification of a hydrolysis method of the sample, the determination of β-linked sugars is important. Also the separative determination of hygroscopic isomaltose and bitter-tasty gentiobiose is important from the standpoint of utilization of a commercial glucose. From these reasons we wish to report herein the

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separative determination of β-linked sugars by emulsin in the oligosaccharides fraction isolated from commercial glucoses by chromatography on a carbon-Celite column.

Previously, we reported on the behavior of sugars (glucose, maltose and maltotriose) for carbon-Celite column chromatography (2) and also in Part 1 of this series, the sugar composition of sweet potato starch hydrol was reported (3). Calculating the recoveries from the later results analyzed by the same method, that of glucose was 98.6 per cent, disaccharides 73.1 per cent, oligosaccharides 35.6 per cent and average was 86.2 per cent on the carbon-Celite column chromatography. The recovery of glucose was very good but that of oligosaccharides was very low. And then, calculating the data in the reports of other researchers, the average recoveries were 80–95 per cent and that of individual sugars were not described. From our results, although the recoveries of oligosaccharides were low, the recoveries of mono- and di-saccharides were considerably good and the average recoveries would probably be 80–95 per cent. In this study, the recoveries of oligosaccharides were 39.2–71.6 per cent and the average was 48.78 per cent. We must therefore, take consideration of oligosaccoarides content obtained from the quantitative determination by the carbon column chromatographic method.

## Experimental

The samples used in this report were the same as in the previous report. Namely, ten samples of the commercial glucoses were used in this studies. Sample of Nos. 1–3 and No. 10 (powdered glucoses) were produced by enzyme hydrolysis, Nos. 4–6 (powdered glucoses) were produced by acid hydrolysis, Nos. 7–9 are crystalline substance produced by acid hydrolysis. All samples were received at the end of 1959 by incorporation through the Institute of Food Research, Department of Agriculture and Forestry.

(I) *Isolation of the Oligosaccharides by Carbon Column Chromatography.*

The oligosaccharides in the samples were isolated by the chromatographic method involving the passage of the solution through a carbon column and subsequent elution of the column with aqueous solution containing increasing concentrations of ethanol. The column was prepared in a glass cylinder measuring 2.5×22 cm with a fritted glass bottom. The adsorbent was made by mechanically mixing 4 g of activated carbon (Takeda) with 4 g of Celite No. 545. A 1 g portion of the samples was brought to a volume of 10 *ml* by distilled water. The solution was then placed on the carbon column. Glucose was removed first by eluting the column with 200 *ml* of water which was sufficient to show the Molisch reaction of glucose negative by the preliminary test. Recovered glucose was determined by the reducing power

calculated as the anhydrous sugar. The oligosaccharides portion of the samples was displaced with 200 *ml* of 35 per cent aqueous ethanol.

The water effluent fraction contained only glucose and no oligosaccharide on a paper chromatography. The oligosaccharides components had been clearly identified on a paper chromatography in the concentrated effluent of ethanol displaced fraction. By this technique, identification of minor sugar in the sample is very easy. Further, very small amounts of glucose were identified very often in such concentrated effluent of ethanol displaced fraction.

The recovered glucose fraction was brought to a volume of 500 *ml* and was determined by the Bertrand-Henmi method : the recovered oligosaccharides fraction was concentrated to a volume of 50*ml* and was determined by Somogyi-reagent after hydrolysis by acid. The obtained result is shown in Table 1.

**Table 1.** Yield of glucose and oligosaccharides by carbon column chromatograpy.

| | | Enzyme hydrolysis | | | Acid hydrolysis | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample No. | | | | | Powder | | | Crystalline | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Total sugar | | 91.11 | 88.20 | 93.46 | 89.76 | 88.75 | 90.68 | 91.30 | 91.68 | 89.64 |
| Reducing sugar | | 88.48 | 85.33 | 92.32 | 81.06 | 82.34 | 81.15 | 90.78 | 90.99 | 88.24 |
| Yield g | Glucose | 85.06 | 82.00 | 87.25 | 75.77 | 76.34 | 72.90 | 92.03 | 90.71 | 85.21 |
| | Oligo-saccharides* | 1.70 | 2.50 | 1.90 | 5.41 | 5.53 | 5.62 | 0.10 | 0.38 | 2.09 |
| | Total | 86.76 | 84.50 | 89.15 | 81.18 | 81.87 | 78.52 | 92.13 | 91.09 | 87.30 |
| Recovering Per cent | Glucose | 97.3 | 100.0 | 97.8 | 97.8 | 97.7 | 95.5 | 101.0 | 99.5 | 99.5 |
| | Oligo-saccharides | 46.5 | 40.4 | 45.6 | 44.0 | 52.1 | 39.2 | 47.6 | 71.6 | 52.1 |
| | Total | 95.2 | 95.8 | 95.4 | 90.4 | 92.2 | 86.5 | 100.9 | 99.3 | 97.4 |

* Values were calculated as glucose.

As is shown in Table 1, the separation of glucose from oligosaccharides has been succeeded but the recovery of oligosaccharides was very low. The total amount of recovered glucose and oligosaccharides was 87–101 per cent for the total gugar which was determined by the Bertrand-Henmi method. Also the recovery of true glucose was 94–101 per cent for the value of true glucose which was determined by the paper chromatographic method. Especially the recoveries of crystalline state samples which contained very small amounts of oligosaccharides (Nos. 7, 8 and 9) were very good, namely 100–101 per cent. On the contrary, that of oligosaccharides were 35–72 per cent, the average was 48 per cent. It is true that recovery of disaccharides was of larger amount than that of higher oligosaccharides but the β-linked disaccharides were contained also in the oligosaccharides fraction on a carbon

column chromatography. Therefore further examination was continued as follows.

The oligosaccharides portions of the three kinds of samples (Nos, 1 2 and 4) were displaced with 50 per cent aqueous ethanol solution after 35 per cent solution. The recoveries were very low as is shown in Table 2.

**Table 2.** The recoveries of oligosaccharides with 35 and 50 per cent aqueous ethanol solution.

| Eluates | | | Sample No. | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 4 |
| A | 200 *ml* of 35% ethanol solution | (g) | 1.70 | 2.50 | 5.41 |
| B | 150 *ml* of 50% 〃 | (g) | 0.03 | 0.28 | 0.03 |
| C | Total | (g) | 1.73 | 2.78 | 5.44 |
| D | B/C 100 | (%) | 1.7 | 10.1 | 0.6 |

* Values in A and B were calculated as glucose.

The oligosaccharides of sample No. 2 were recovered about 10 per cent by 50 per cent aqueous ethanol solution for the amount which was recovered by 35 per cent aqueous ethanol solution. This might be owing to the presence of a large amount of higher oligosaccharides in the sample No. 2 as is shown on PPC (1).

*(II) The Yielding of Sugars by Carbon Column Chromatography.*

Further examinations of yielding of sugars were carried out. 0.9930 g of sample No. 10 and 0.9880 g of another new sample No. 11 which were hydrolyzed by enzyme preparation were brought to a volume of 10 *ml*. The solution was then placed on the carbon-Celite column as described above. The sugars were removed by eluting the column with water (200 *ml*), 5 (100 *ml*), 10 (100 *ml*), 15 (100 *ml*), 20 (100 *ml*), 25 (100 *ml*) and 30 per cent (200 *ml*) aqueous ethanol solution. Glucose in water effluent fraction was determined by the Bertrand-Henmi method. Oligosaccharides in 5–30 per cent aqueous ethanol fraction were hydrolyzed by 2.27 per cent HCl for 2.5

**Table 3.** The recoveries of sugars by carbon column chromatography.

| Effluent | Volume *ml* | Sample No. | | | |
|---|---|---|---|---|---|
| | | 10 | | 11 | |
| | | Yield g | Per cent | Yield g | Per cent |
| Water | 200 | 0.7608 | 76.588 | 0.8120 | 82.186 |
| 5% Ethanol | 100 | 0.0035 | 0.352 | 0.0056 | 0.567 |
| 10 〃 | 100 | 0.0238 | 2.396 | 0.0154 | 1.558 |
| 15 〃 | 100 | 0.0189 | 1.902 | 0.0126 | 1.278 |
| 20 〃 | 100 | 0.0105 | 1.059 | 0.0056 | 0.567 |
| 25 〃 | 100 | 0.0077 | 0.775 | 0.0056 | 0.567 |
| 30 〃 | 200 | 0.0070 | 0.706 | 0.0063 | 0.639 |

hours and were determined by Somogyi reagent after neutralization. The result is shown in Table 3.

The yielding of recoveries which were calculated from Table 3 and the above described paper chromatographic analysis are shown in Table 4.

**Table 4.** The per cent of recoveries of glucose and oligosaccarides by carbon column chromatography.

| | No. 10 | No. 11 |
|---|---|---|
| A Glucose (determined by paper chromatographic analysis) | 75.65% | 80.25% |
| B Oligosaccharides (ibid) | 13.31 | 10.27 |
| C Glucose (recovered by carbon column chromatography) | 76.59 | 82.19 |
| D Oligosaccharides (ibid) | 7.14 | 5.18 |
| Recovered per cent { Glucose C/A | 100.56 | 101.18 |
| Recovered per cent { Oligosaccharides D/A | 54.02 | 50.44 |

At the first experiment (Table 1), 200 *ml* of water and 200 *ml* of 30 per cent aqueous ethanol solution were used as the developer. At the second experiment (Table 4), gradient elution method which involved the passage of 900 *ml* of water and aqueous solution containing increasing concentration of ethanol was applied to remove the adsorbed sugars. In both methods, the recoveries of glucose were almost 100 per cent but that of oligosaccharides were increased to almost 15 per cent and was yet only 50–54 per cent of the paper chromatographic analysed values. By carbon column chromatography, the recovery of higher oligosaccharides which was displaced with 25–30 per cent aqueous ethanol solution was almost 1.5 per cent (No.10) and almost 1.2 per cent (No. 11) in comparison with that the former was 5.9 per cent and the later was 4.4 per cent by paper chromatographic analysis. Namely only 36–39 per cent of the higher oligosaccharides was recovered. These less recovery of oligosaccarides might be due to that they remained in a carbon column without being displaced with aqueous ethanol solution. Further examination will be continued.

*(III) The Determination of β-Linked Sugars by Emulsin.*

(i) The determination of β-linked sugars in total oligosaccharides

By emulsin (β-glucosidase) the separative determination of β-linked sugars in the mixture of α- and β-linked sugars was carried out. Emulsin was prepared from almond by the modification of the Tauber method.

0.1 Grame of crude emulsin was dissolved in 5 *ml* of acetate buffered solution (pH=4.7). Equal or two times the buffered solution was added to 1 per cent of gentiobiose solution and the subsequent solution was incubated at 30°C. It was demonstrated by paper chromatography that after for 4 or 2 days, gentiobiose was hydrolyzed to glucose and then no glucose was formed

from $\alpha$-linked isomaltose or buffered emulsin solution itself by the same method.

The oligosaccharides fraction isolated by carbon column chromatography was evaporated to dry in vacuo, and was dissolved in 0.3 *ml* of water. 0.05 *ml* of the solution was added to 0.1 *ml* of buffered emulsin solution and was incubated for 4 days at 30°C. As above described, there are small amounts of glucose in the oligosaccharides fraction and after digestion with emulsin there remained many kinds of $\alpha$-linked disaccharides and higher oligosaccharides. Therefore those sugars were determined quantitatively by the paper chromatographic method as follows : Aliquots of concentrated or digested solution was applied to a filter paper sheet (20×40 cm) and developed as in the previous report. Locate the respective sugars and excise appropriate sections of the chromatogram. After extraction of sugar from each sections, the determination was made by Somogyi reagent as is shown in Table 5. In Table 5, A is the contaminated glucose in the oligosaccharides fraction, B is the amount of glucose after digestion by emulsin, C(B-A) is the amount of glucose produced by digestion of $\beta$-linked sugars with emulsin and D is per cent for total oligosaccharides.

**Table 5.** Digestion of oligosaccharides by emulsin.

| | Sample No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | Glucose blank % | — | — | 0.02 | 0.05 | 0.05 | 0.10 | 0.00 | 0.00 | 0.04 |
| B | Glucose after digestion % | — | — | 0.29 | 3.20 | 2.87 | 2.77 | 0.06 | 0.29 | 1.29 |
| C | B-C | — | — | 0.27 | 3.15 | 2.83 | 2.67 | 0.06 | 0.29 | 1.25 |
| D | C/total oligosaccharides×100 | — | — | 6.5 | 25.6 | 26.7 | 18.6 | 29.0 | 54.5 | 31.2 |

(ii) The determination of gentiobiose and isomaltose

The presence of $\beta$-linked sugars are a marked difference owing to the preparation method of glucose and the content of gentiobiose which has a bitter taste is an important mark for utilization of a commercial glucose. Thus gentiobiose which was overlapped with isomaltose was extracted from their paper chromatogram. After digestion by emulsin, the separative determination was carried out. Aliquots of 0.3 *ml* of the 20 per cent sample solutions were applied to a Toyo No. 2 filter paper sheet. Paper chromatogram was made as already described. Gentiobiose together with isomaltose was extracted from it and was evaporated to dry in vacuo. After then, 0.3 *ml* of emulsin acetate buffer solution was added and was incubated for 3 days at 30°C. After digestion, all of the solution was spotted to a filter paper sheet. Glucose which was produced from $\beta$-linked gentiobiose by emulsin and isomaltose was extracted from chromatogram. The ratio of each sugar was determined by the Somogyi reagent. Gentiobiose and isomaltose was calcu-

lated as shown in Table 6 from this ratio and from their total content was shown in the previous report.

**Table 6.** Separative determination of isomaltose and gentiobiose by emulsin.

| | | Enzyme hydrolysis | | | | Acid hydrolysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sample No. | 1 | 2 | 10 | 11 | 4 | 5 | 6 | Average |
| A | Gentiobiose | 0 | 0 | 0 | 0 | 2.43 | 1.90 | 1.93 | 2.09 |
| B | Total | 1.94 | 1.60 | 1.81 | 0.26 | 5.74 | 6.64 | 5.93 | 6.10 |
| C | Isomaltose (B-A) | 1.94 | 1.60 | 1.81 | 0.26 | 3.31 | 4.74 | 4.00 | 4.01 |
| D | A/B×100 | 0 | 0 | 0 | 0 | 42 | 29 | 33 | 34 |
| E | Isomaltose/Gentiobiose (C:A) | — | — | — | — | 6:4 | 7:3 | 7:3 | 6.6:3.4 |

* Values were calculated as glucose.

## Discussion

In the commercial glucoses a large part of sugar is glucose, but some amount of oligosaccharides is contained. For their isolation and determination carbon column chromatography is effective. We obtained good results on the separative determination of glucose, maltose and maltotriose (2). Carbett reported on the separative determination of mono- and disaccharides by carbon column chromatography (4). Patterson *et al.* reported on the separative determination of glucose and maltose in a starch hydrolysate using the same method (5). In the 11th meeting of ICUMSA at Paris 1954, representationes of England proposed that carbon column chromatography should be applied to the standard procedure of analysis of starch hydrolysates (6).

In this report oligosaccharides in commercial glucoses were isolated by carbon column chromatography. The recovery of glucose was almost perfect but that of oligosaccharides was low. Evidence in the above literatures and in our earlier reports (2, 3) indicates that the recovery of disaccharides such as gentiobiose and isomaltose is somewhat good but we must take care about the low recovery of higher oligosaccharides. The oligosaccharides which were isolated from powdered samples hydrolyzed by acid with carbon column chromatography were digested by emulsin as 19–27 per cent (average 24 per cent) and that of crystalline samples hydrolyzed by acid were digested as 29–55 per cent (average 38 per cent). The oligosaccharides which were isolated from the two in three samples hydrolyzed by enzyme preparation, were did not digested but the one was only digested as 6 per cent. This reason can not be explained because the preparation method was not known distinctly. If there was trace α-glucosidase in the used emulsin, the oligosaccharides of the other two samples should be digested. Also the exact presence of β-linkage in starch has not been reported. We have no informa-

tion as to whether in this sample acid liquification was applied. Peat *et al.* reported the formation of both $\alpha$- and $\beta$-linked sugars from glucose by enzyme preparation of *Aspergillus niger* (7). Enzyme preparation which was used in manufacture was prepared from *Rhizopus sp.* and could digest starch as much as 100 per cent. Further examination will be continued.

On the oligosaccharides which were isolated by carbon column chromatography, the recovery, must be checked. Since the separative determination of gentiobiose and isomaltose is an important point among them it was carried out by paper chromatographic method. Locate gentiobiose spot overlapped isomaltose and excise appropriate sections of the paper chromatogram. After extraction and digestion by emulsin the separative determination was made. From the result (Table 6), 1.9–2.4 per cent (average 2.1 per cent) was digested by emulsin and corresponded to the content of gentiobiose; not digested portion was 3.3–4.7 per cent (average 4.0 per cent) and was isomaltose. But isomaltose fraction which was prepared from the samples hydrolyzed by enzyme preparation was not digested by emulsin. The average content of oligosaccharides of the three samples produced by acid hydrolysis is 12.4 per cent as shown in Table 1 of the previous report. Gentiobiose which was calculated from glucose formed by emulsin is 17 per cent. Isomaltose which was calculated from remained gluco-biose is 32 per cent. From the earlier reports of Montgomery *et al* (8) (Carbohydrate composition of hydrol) and of Peat *et al.* (9) (Glucose reversion products), the contents of isomaltose and gentiobiose were calculated as shown in Table 7 (8) (9).

**Table 7.** Content of isomaltose and gentiobiose.*

| | Hydrol (Montgomery *et al.*) | Glucose reversion (Peat *et al.*) | Hydrol (Authors) |
|---|---|---|---|
| Gentiobiose | 14 | 23 | 17 |
| Isomaltose | 41 | 34 | 32 |
| Total | 55 | 57 | 49 |

* Values are per cent for oligosaccharides.

The contents of isomaltose and gentiobiose depend on and raw starch hydrolyzed conditions (kind and concentration of acid, temperature, pressure, hour and etc). Also the contents reported by Montgomery *et al.* were calculated from the yields of crystalline octaacetates. Namely calculated values are different by researchers. Generally, 50–60 per cent of oligosaccharides were 1,6-linked isomaltose and gentiobiose, and among them almost 30 per cent was isomaltose and almost 20 per cent was gentiobiose. Thompson *et al.* demonstrated that the ratio of isomaltose and gentiobiose in the acid reversion products of glucose was 1 : 1 and isomaltose content is 200 times of gentiobiose in the acid hydrolysate of amylopectin (10). They concluded

that isomaltose was derived from the branched point of amylopectin. Peat *et al* reported on the determination of reversion products of glucose as is in Table 8 (9). The ratio of isomaltose and gentiobiose was 6 : 4. And from our results it was 6.6 : 3.4 (Average of three samples).

**Table 8.** Products of reversion of glucose by Acid. (Glucose (1.0 %) heated in 0.33 N-$H_2SO_4$ for 10 hrs)

| | |
|---|---|
| Disaccharides | 0.307% |
| Isomaltose | 0.105 |
| Gentiobiose | 0.071 |
| Nigerose | 0.025 |
| Laminaribiose | 0.008 |
| Maltose + Cellobiose | 0.054 |
| Kojibiose + Sophorose + Trehalose | 0.044 |


## Summary

By using emulsin, the determination of β-linked sugar contents were taken on the commercial glucoses produced by acid or enzyme hydrolysis of starch. The oligosaccharides fraction of commercial glucoses produced by acid hydrolysis were hydrolyzed remarkably with emulsin. This fact shows the presence of such β-linked sugars as gentiobiose and then remarkable difference on the two type samples. The average content of total oligosaccharides of the commercial powdered glucoses produced by acid hydrolysis was 12.4 per cent and almost half (6.1 per cent) of the total oligosaccharides was 1,6-linked disaccharides, in which isomaltose and gentiobiose were 4.0 and 2.1 per cent respectively. The oligosaccharides fraction of some commercial powdered glucoses produced by enzyme preparation was slightly hydrolyzed with emulsin, but its digested amount was too small to prove the presence of β-linked sugars, and further examination will be carried out.



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