Presence of Levoglucosan in Cornstarch Hydrolyzates

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Levoglucosan (1,6-anhydro-D-glucose) was found to the extent of 2.3% in a quantitative paper chromatographic investigation of cornstarch acid-hydrolyzates at equilibrium. The identification of levoglucosan in constant amount supports the view that the cornstarch molecule cannot be hydrolyzed completely to D-glucose by acid. In this study, other components tentatively identified were 5-hydroxymethyl-2-furaldehyde, glycerol, xylose, arabinose, fructose, isomaltose, and gentiobiose. D-Glucose and levoglucosan, when subjected to reaction conditions similar to those for the acid hydrolysis of starch, produce essentially the same equilibrium mixture as that obtained with starch.

THE STUDY OF THE COMPOSITION of acid-hydrolyzed cornstarch systems has received the attention of many investigators (*10*). By various methods of analysis (*10, 12*) these systems have been shown to contain, in addition to D-glucose, a number of other constituents: maltose, gentiobiose, 5-hydroxymethyl-2-furaldehyde (HMF), isomaltose, α,α-trehalose, and levulinic and formic acids. With the advent of methods employing paper chromatography (*6, 11, 15, 16*), a new analytical procedure was made available for study of composition of cornstarch acid hydrolyzates.

This paper presents a study of equilibrated cornstarch acid hydrolyzates by means of quantitative paper chromatography. For the first time 1,6-anhydro-D-glucose (levoglucosan) was found to be present and, in all cases, to the extent of 2.3% on a total carbohydrate basis. Besides its quantitative evaluation by paper chromatography, levoglucosan was isolated on a cellulose column, the triacetate prepared, and its identity verified by a mixed melting point with known material (*13*).

With less certainty, other components were detected—that is, compounds were not isolated and derivatized, but were chromatographically compared with known materials, and further identified by differentiating spray reagents where possible. These included hydroxymethylfuraldehyde, glycerol, xylose, arabinose, fructose, isomaltose, and gentiobiose. Other oligosaccharides were qualitatively detected and quantitatively determined.

Because of the formation of levoglucosan, it was shown that cornstarch, even in dilute solutions, cannot be hydrolyzed completely to D-glucose. Levoglucosan, when subjected to reaction conditions similar to those for hydrolyzing starch, results in essentially the same equilibrium mixture as that produced from starch. This also holds true for the parallel reaction of D-glucose. It can be postulated that carbohydrates derived from D-glucose, whether anhydride or polymer, when subjected to the conditions typical for starch hydrolysis, will produce identical equilibrium mixtures.

These findings agree with those of others in that, at equilibrium, as the concentration of starch is increased, the D-glucose concentration decreases and the oligosaccharide concentration increases (*7, 10*). Likewise in agreement with the reports of other workers, the rate of hydrolysis was found to change when the concentration of acid was altered, other conditions remaining constant (*10*). However, when the acid concentration was changed, no difference was detected in the equilibrium compositions.

The reactions for this study were carried out in both factory and laboratory equipment. The results showed that the size and materials of construction did not affect the composition of the mixtures.

Preparation of Samples

Factory hydrolyzates were made in bronze converters operated at 50 pounds per square inch steam pressure (147° C.). The variables were:

The starch concentration was varied from 0.75 to 31.9%, with the hydrochloric acid catalyst constant (0.028 to 0.029*N*).

The hydrochloric acid catalyst was varied from 0.016 to 0.046*N* with starch concentration constant (14.7%).

Samples, taken at intervals varying from 2 to 4 minutes, were partially neutralized with sodium carbonate solution and later adjusted to pH 4.5 to 4.7. All samples were filtered with asbestos filter aid, but were not decolorized. Phenylmercuric acetate, 1 mg. per liter, together with refrigeration, was used to preserve the portions retained for paper chromatographic analysis.

Laboratory preparations were made in either a small laboratory converter or a small tantalum bomb. For these preparations, 17.4% dry substance, as starch, and 0.033*N* acidity levels were employed. These conditions were the same as the starting conditions for hydrolysis 3, Table I. In the factory these conditions change because of dilution caused by condensation of the injected steam used for heating. The majority of the condensation occurs while the converter approaches full pressure (7 to 8 minutes). The increase in volume amounted to 520 ± 15 gallons (*9*). All samples were taken after the converter reached full pressure.

In the laboratory, starch was hydrolyzed and dextrose and levoglucosan were reverted in the bomb. Dextrose only was reverted in the laboratory converter. Dilution by condensation of steam does not occur in the bomb, but does occur in the laboratory converter. Complete reaction conditions are given in Table I.

Analytical Methods

A large number of factory samples were taken. Dry substance was estimated by the Brix hydrometer method. Dextrose equivalent (D.E.), corrected to salt-free basis, was determined by the Lane-Eynon titration. Sodium chloride was determined by Mohr's method. Color measurements were made with the Lovibond Tintometer (Caramel Series No. 52) and expressed on the same dry substance basis (18 Brix) and cell length (5 inches).

Components detected on chromatograms were compared with known materials and further identified by differen-

tiating spray reagents where possible.

Quantitative paper chromatography was based on the method of Dimler and others (*6*). The solvent was ethyl acetate–pyridine-water (8:2:1 v./v.) (*16*). Whatman No. 1 filter paper was twice washed with water by the descending technique and dried. Development followed in the same manner. All samples were given a "short" development (4 to 6 hours), a period sufficient for the solvent to reach within 2 to 3 cm. of the bottom of the sheet and long enough for the resolution of components migrating faster than fructose. Certain of the same samples were subjected to a "long" development (approximately 24 hours), which separated fructose and D-glucose and partially resolved those components migrating at rates slower than D-glucose. During this development, solvent drips from the serrated lower edge of the paper.

Locator strips were sprayed with ammoniacal silver nitrate (*6*) or urea–phosphoric acid (*17*) reagent. The silver nitrate–sprayed strips were allowed to react at room temperature in the dark. Glucose and isomaltose-gentiobiose areas could be detected within 30 minutes after spraying. Detection of hydroxymethylfuraldehyde, levoglucosan, xylose, and fructose required a longer time—from 6 to 16 hours.

Urea–phosphoric acid–sprayed strips were heated at 100° to 110° C. by the method described by Fetzer and Ough (*8*). Hydroxymethylfuraldehyde showed quickly. Fructose appeared more slowly. Often a characteristic color would show in the disaccharide and higher oligosaccharide areas.

Located components (or groups of components), after being eluted, were quantitatively measured with anthrone (*6*). A 4-hour elution was used. Anthrone solution, freshly prepared for each set of measurements, was dispensed with a 6-ml. Machlett automatic pipet. Measurements were made in triplicate, including a reagent blank which was measured against water. The median reagent blank was used as the reference standard, and the median value of all other measurements was used in the calculations (*4*). A Coleman Universal Model 14 spectrophotometer was employed.

A paper control and solution control were simultaneously determined for comparative purposes. The paper control was a section of blank filter paper from the developed chromatogram, on which a duplicate volume of sample was placed and then eluted in the same manner as sections bearing located components. The solution control consisted of a duplicate volume of sample, which was placed directly in a volumetric flask of the proper size.

Because the amounts of components migrating in advance of glucose were small, and reducing substances eluted from filter paper itself are not uniformly distributed, filter paper corrections were determined as closely as possible.

Discussion

Dextrose equivalent–time curves for the factory hydrolyzates leveled off at constant values, as shown in Figures 1 and 2. The samples for paper chromatographic analysis were taken from those represented by the flat portion of the curves. In the case of hydrolysis 1, Table I, made with 0.75% starch, the dextrose equivalents were not calculated because of the extreme dilution. Instead, samples were chosen from those having constant Lane-Eynon titration values.

The results of the Lane-Eynon determinations indicated different equilibria for the different starch concentrations. This was substantiated by the paper chromatographic determinations as the results of the "short" developments show for the six factory hydrolyses (Table II).

This is also shown in the results obtained with the "long" developments (Table III). In order to take into account the materials that are swept from the paper during the long development, the values in Table I were calculated by proportioning the glucose-fructose and oligosaccharides in the short developments (Table II) and applying these ratios.

The variation in analytical values for the plateau samples falls within the limits of precision for the method (*6*). Therefore an equilibrium is reached if hydrolyses are carried out for a long enough time. The median percentages (Table II) can be considered the equilibrium values for the components (groups of components) produced by the hydrolysis conditions.

The median values for levoglucosan, D-glucose–fructose, and oligosaccharides under various conditions of starch concentration are plotted in Figure 3. The levoglucosan percentage remained constant. Levoglucosan is formed by intramolecular dehydration of glucose.

Table I. Reaction Conditions and Analytical Results

Hydrolysis or Reaction	1	2	3	4	5	6	7	8	9	10
Material reacted	Starch	Starch	Starch	Starch	Starch	Starch	Starch	Levoglucosan	D-Glucose	D-Glucose
% dry substance	0.75	7.0	14.9	31.9	14.7	14.7	17.4	17.4	17.4	17.4
Reaction vessel	Factory	Factory	Factory	Factory	Factory	Factory	Lab. bomb	Lab. bomb	Lab. bomb	Lab. conv.
Volume, calcd., gal.	3170	3380	3380	3380	3380	3380	...	...	...	...
20.1° Bé HCl used, lb.	90[a]	90[b]	90[b]	90[b]	53 1/4[b]	150[b]	...	...	...	...
Normality, calcd.	0.029	0.028	0.028	0.028	0.016	0.046	0.033	0.033	0.033	0.033
Equilibrium D.E.[c]	[d]	93.9	93.0	86.8	92.0	92.0	...	...	...	...
Short development[e]										
HMF	0.25	0.94	0.66	0.67	0.71	0.77	[f]	[f]	0.2	0.8
Levoglucosan	2.0	2.3	2.4	2.4	2.6	2.5	2.3	2.0	2.2	2.2
Xylose[g]	0.13	[f]	0.11	0.34	0.07	[f]	[f]	[f]	0.1	0.1
Glucose, fructose	95.5	91.9	87.1	76.2	87.1	86.9	84.8	85.7	86.2	87.8
Oligosaccharides	2.1	4.9	10.1	20.3	9.5	9.9	12.9	12.3	11.3	9.0
Long development[e]										
Fructose	...	0.01	0.22	0.02	...	...	...	...	...	...
Glucose	...	91.9	86.9	76.2	...	...	...	...	...	...
Maltose, etc.	...	1.5	3.1	6.2	...	...	...	...	...	...
Isomaltose, etc.	...	3.2	6.1	10.1	...	...	...	...	...	...
Higher sugars	...	0.22	0.9	4.0	...	...	...	...	...	...

[a] 19.9° Bé. HCl added directly to starch suspension.
[b] Added to 200 gal. water and charged into reaction vessel before starch was introduced.
[c] Corrected to salt-free basis.
[d] Constant Lane-Eynon titration used as equilibrium.
[e] % as glucose.
[f] Detectable but not quantitatively determinable.
[g] Values indicate order of magnitude.

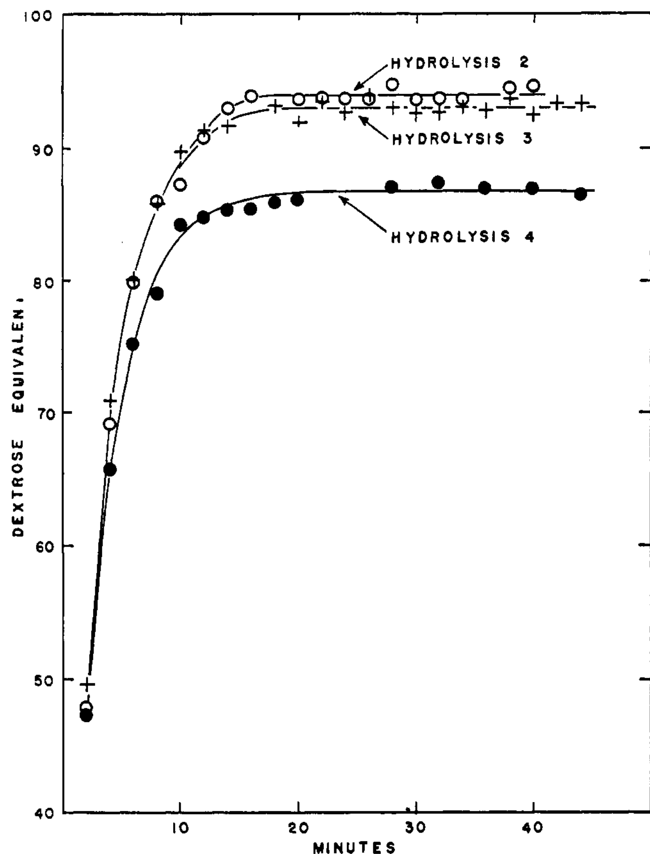


Figure 1. Effect of starch concentration on dextrose equivalent with acid catalyst constant

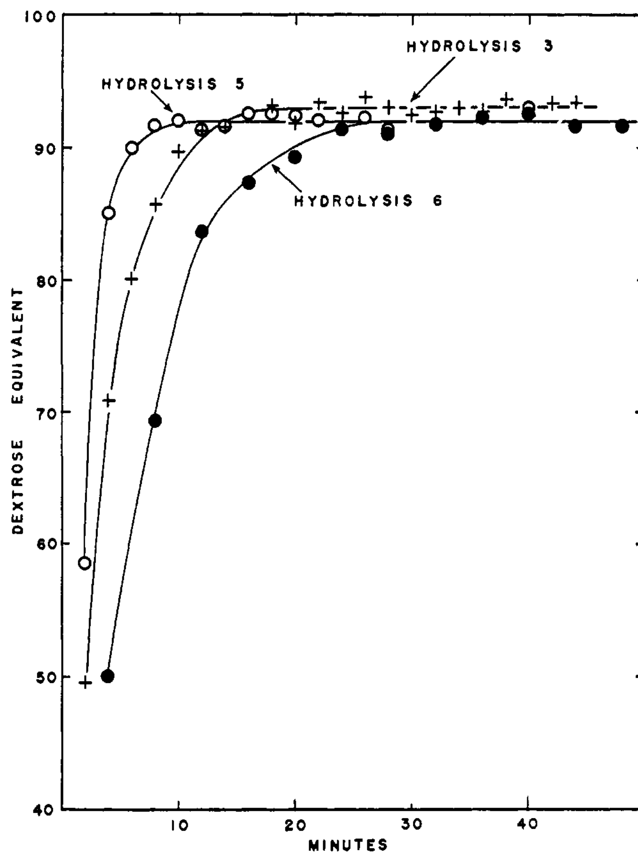


Figure 2. Effect of acid concentration on dextrose equivalent with starch concentration constant

It is possible that oligosaccharides undergoing a similar dehydration process followed by hydrolysis could also form levoglucosan. As expected, when the starch concentration increased, D-glucose-fructose percentages decreased and oligosaccharide percentages increased (*7, 10*).

Extrapolating the D-glucose–fructose curve (Figure 3) to zero starch concentration, or infinite dilution, shows that starch cannot be completely hydrolyzed to D-glucose. By the method of least squares, the extrapolated value is 96.2%. The determined fructose differences change this value very little. Using the D-glucose values from the long developments (Table I) and the method of least squares, the extrapolated value for D-glucose is 96.3%. Other workers in this field have also suggested this to be the case. Peckham and Engel (*14*), working at 0.25% concentrations of both starch and sugar mixtures, found a correction factor to be necessary to obtain correct analytical values. Dimler and others (*5*), working with dextran, have also found this to be true. This would be expected if the levoglucosan concentration is constant in these equilibrium mixtures.

Levoglucosan was recovered in these laboratories (*13*) on a cellulose column from a cornstarch hydrolyzate. The triacetate melted at 109–110°, and a mixed melting point with levoglucosan triacetate, prepared in the laboratory of M. L. Wolfrom at Ohio State University, showed no depression. Other work in these laboratories indicates that the presence of D-glucose has a strong inhibiting effect on the crystallization of levoglucosan. This may be the reason why levoglucosan has not previously been isolated directly from starch hydrolyzates.

Dehydration is also responsible for the formation of hydroxymethylfuraldehyde from D-glucose. Except for hydrolyses 1 and 2, Table II, the median percentages of hydroxymethylfuraldehyde are fairly constant. Hydroxymethylfuraldehyde decomposes in part into formic and levulinic acids, which may be the reason for the differences.

The presence of xylose and arabinose was detected. The quantity of xylose may be too small to be precisely determined with anthrone under the conditions employed. On an equal weight basis, Dimler and others (*6*) reported the relative absorbance obtained with xylose to be but 7% of that obtained with glucose. This relationship has been verified with National Bureau of Standards standard dextrose and D-xylose, the phenylosazone of which melted at 162–164°. Single spots only were observed by paper chromatography with the known xylose. On this basis, the xylose percentage could be larger than is apparent.

The presence of arabinose can be rationalized as a reverse aldolization of D-glucose. The presence of xylose and glycerol in the starch hydrolysis could arise from the small quantities of cellulose fiber and fat that occur in starch. In addition, starch itself may be partially carboxylated at carbon atom 6, or this position may be oxidized during the hydrolytic process. Subsequent hydrolysis and decarboxylation of an oxidized product would also account for the presence of xylose.

Further work was done to determine whether xylose and glycerol were present in the reaction products from dextrose and levoglucosan. As these concentrations are very small, it is necessary to place a large quantity of material on a chromatogram, elute the section under study, concentrate, and rechromatogram the concentrate before identifying the resulting spots.

This has been done, taking into account the effect of contaminants from the paper. Although there is suggestive evidence that a very small quantity of glycerol is present in the reactions of dextrose and levoglucosan, it has not been detected in the quantity in which it is present in cornstarch hydrolysis. In the dextrose and levoglucosan reversions, an area coincided with the location of known xylose. Instead of the characteristic pentose color with aniline–phosphoric acid, a light yellow color developed. This suggests a different component. It could be present in the

starch hydrolysis reaction and be masked by the xylose which is shown to be present. Therefore, the values (Table I) shown for xylose might be contributed by the unknown component.

The long developments separated fructose from D-glucose and separated the oligosaccharides into three groups. Qualitative tests by the Bayly and Bourne (*7*) procedure with the second group have indicated the presence of isomaltose and gentiobiose. One might expect the first group to contain maltose; the third, panose.

Fructose was present in small amounts. Its presence suggests that the Lobry de Bruyn–Alberda Van Eckenstein transformation occurs to a slight extent in acid solution. Berner and Sandlie (*2*) have reported the formation of fructose from glucose by boiling it in water and in 0.5*N* sulfuric acid solution. Its presence was detected in unneutralized D-glucose reversion liquor. Although in all other samples its presence could be accounted for as having resulted from local overneutralization, this need not be the case. Alkalinity due to pyridine in the solvent was suggested as a possible cause for the formation of fructose from D-glucose during development. When D-glucose was developed under the same conditions as the equilibrated samples, fructose was not detected. Moreover, fructose was found in equilibrated samples when solvents not containing pyridine were used.

Reactions employing dextrose or levoglucosan resulted in the same equilibrium mixtures as the starch reactions. Any carbohydrate derived of dextrose, whether anhydride or polymer, and subjected to the conditions of starch hydrolysis, should result in an identical mixture.

In hydrolyses 5 and 6, the concentration of acid used was varied from the amount normally employed in factory

Table II. Short Development Analyses

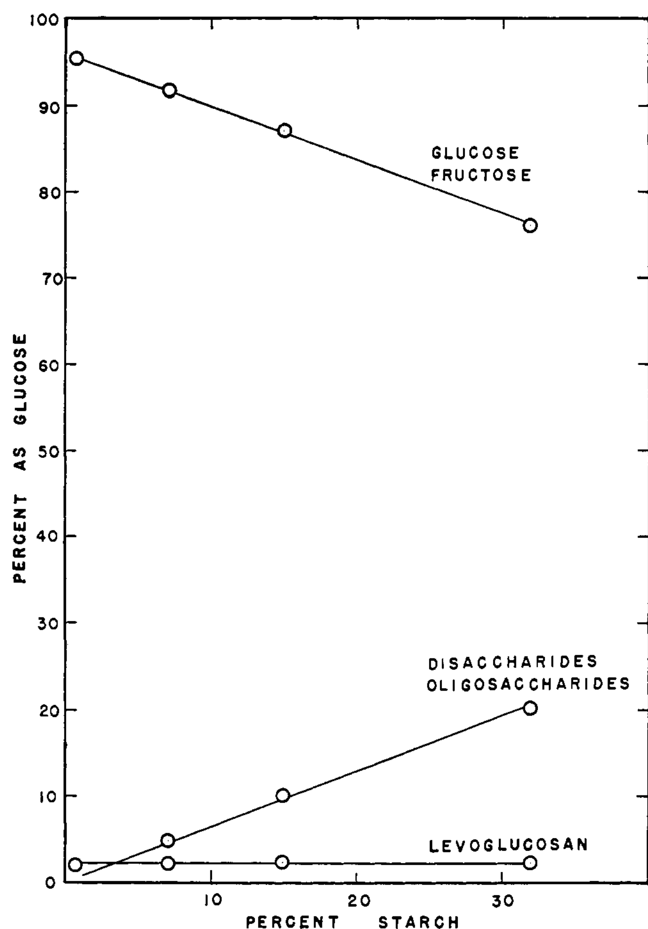
Hydrolysis	Component	Minutes: 14	16	18	20	22	24	26	28	32	34	36	40	44	48	Median
		Per Cent as Glucose														
1	HMF	...	...	0.34	...	0.16	...	...	...	...	...	...	...	...	...	0.25
	Levoglucosan	...	...	2.1	...	1.9	...	...	...	...	...	...	...	...	...	2.0
	Xylose[a]	...	...	0.25	...	[b]	...	...	...	...	...	...	...	...	...	0.13
	Glucose, fructose	...	...	95.2	...	95.9	...	...	...	...	...	...	...	...	...	95.5
	Oligosaccharides	...	...	2.1	...	2.1	...	...	...	...	...	...	...	...	...	2.1
2	HMF	...	...	...	...	0.16	...	...	...	1.1	0.89	...	0.99	...	...	0.94
	Levoglucosan	...	...	...	...	2.2	...	...	...	2.8	2.5	...	2.1	...	...	2.3
	Xylose[a]	...	...	...	...	[b]	...	...	...	0.10	[b]	...	[b]	...	...	[b]
	Glucose, fructose	...	...	...	...	91.7	...	...	...	91.0	92.0	...	92.0	...	...	91.9
	Oligosaccharides	...	...	...	...	6.0	...	...	...	5.0	4.7	...	4.9	...	...	4.9
3	HMF	...	0.43	[b]	0.69	...	...	0.63	...	...	0.77	...	...	0.77	...	0.66
	Levoglucosan	...	2.5	2.1	2.6	...	...	2.3	...	...	2.1	...	...	2.7	...	2.4
	Xylose[a]	...	0.13	[b]	[b]	...	...	0.10	...	...	0.31	...	...	0.34	...	0.11
	Glucose, fructose	...	86.0	87.1	86.2	...	...	87.9	...	...	87.2	...	...	87.5	...	87.1
	Oligosaccharides	...	11.0	10.8	10.5	...	...	9.1	...	...	9.6	...	...	8.7	...	10.1
4	HMF	...	...	...	...	...	0.69	...	0.90	...	...	0.66	...	0.51	...	0.67
	Levoglucosan	...	...	...	...	...	2.5	...	2.3	...	...	2.6	...	2.2	...	2.4
	Xylose[a]	...	...	...	...	...	0.29	...	0.42	...	...	0.29	...	0.39	...	0.34
	Glucose, fructose	...	...	...	...	...	76.1	...	75.9	...	...	76.3	...	76.6	...	76.2
	Oligosaccharides	...	...	...	...	...	20.4	...	20.5	...	...	20.2	...	20.3	...	20.3
5	HMF	...	...	...	...	...	...	...	...	0.48	...	...	...	...	0.94	0.71
	Levoglucosan	...	...	...	...	...	...	...	...	2.3	...	...	...	...	2.9	2.6
	Xylose[a]	...	...	...	...	...	...	...	...	[b]	...	...	...	...	0.13	0.07
	Glucose, fructose	...	...	...	...	...	...	...	...	87.1	...	...	...	...	87.1	87.1
	Oligosaccharides	...	...	...	...	...	...	...	...	10.1	...	...	...	...	8.8	9.5
6	HMF	0.44	...	...	...	...	...	1.1	...	...	...	...	...	...	...	0.77
	Levoglucosan	2.5	...	...	...	...	...	2.6	...	...	...	...	...	...	...	2.5
	Xylose[a]	[b]	...	...	...	...	...	[b]	...	...	...	...	...	...	...	[b]
	Glucose, fructose	87.2	...	...	...	...	...	86.5	...	...	...	...	...	...	...	86.9
	Oligosaccharides	9.9	...	...	...	...	...	9.8	...	...	...	...	...	...	...	9.9

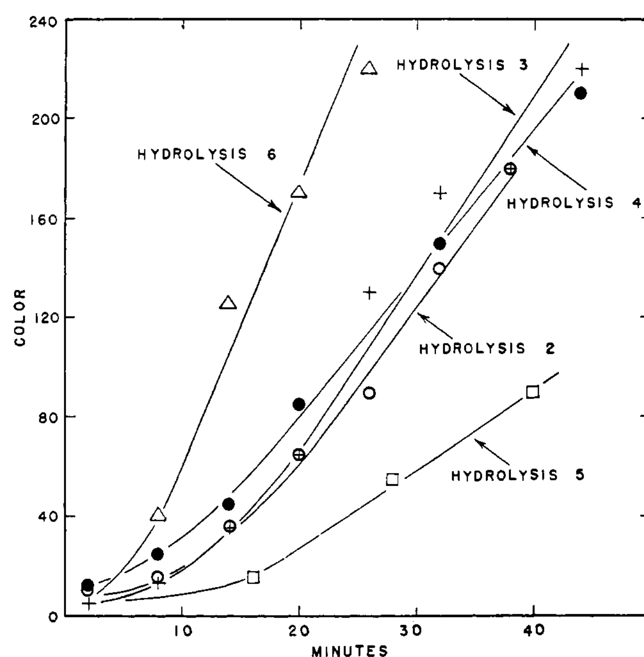
[a] Values indicate order of magnitude.
[b] Detectable but not quantitatively determinable.

Table III. Long Development Analyses

Hydrolysis	Component	Minutes: 16	18	20	22	24	26	28	32	34	36	40	44	Median
		Per Cent as Glucose												
2	Fructose	...	...	...	0.01	...	...	...	[a]	[a]	...	0.45	...	0.01
	Glucose	...	...	...	94.3	...	...	...	95.3	94.9	...	95.1	...	95.0
	Maltose, etc.	...	...	...	1.8	...	...	...	1.3	1.6	...	1.1	...	1.5
	Isomaltose, etc.	...	...	...	3.7	...	...	...	3.2	3.3	...	3.2	...	3.3
	Higher sugars	...	...	...	0.25	...	...	...	0.24	0.22	...	0.17	...	0.23
3	Fructose	0.22	0.65	0.41	...	...	0.24	...	...	0.19	...	...	0.11	0.23
	Glucose	88.7	89.0	89.9	...	...	89.5	...	...	89.8	...	...	90.1	89.7
	Maltose, etc.	3.4	3.1	3.1	...	...	2.5	...	...	3.5	...	...	2.9	3.1
	Isomaltose, etc.	6.4	6.3	5.7	...	...	6.1	...	...	5.6	...	...	6.0	6.1
	Higher sugars	1.2	0.9	0.9	...	...	1.2	...	...	0.9	...	...	0.9	0.9
4	Fructose	...	...	...	...	0.12	...	0.04	...	...	[a]	...	[a]	0.02
	Glucose	...	...	...	...	78.0	...	78.9	...	...	79.0	...	80.3	78.9
	Maltose, etc.	...	...	...	...	7.3	...	6.8	...	...	6.0	...	5.7	6.4
	Isomaltose, etc.	...	...	...	...	10.3	...	10.2	...	...	11.0	...	10.4	10.3
	Higher sugars	...	...	...	...	4.2	...	4.1	...	...	4.0	...	3.5	4.1

[a] Detectable but not quantitatively determinable.





▲ Figure 4. Color development during acid hydrolysis of cornstarch

◀ Figure 3. Component varia tion of cornstarch hydrolyzates at hydrolysis equilibrium with change in starch concentration

practice. The conditions and results of this experiment are shown in Tables I and II by comparing hydrolyses 3, 5, and 6. It can be seen that, in the acid range used for the experiment, neither the dextrose equivalent nor the components in the equilibrium mixtures of the hydrolyses were influenced by the acid concentration.

Some workers (*3*) have reported that in starch hydrolysis the D-glucose content decreases soon after the maximum dextrose equivalent has been reached. For the hydrolyses reported here, neither the dextrose equivalent nor the D-glucose–fructose percentages decreased during the times studied, which are much longer than normal factory practice.

Figure 4 shows the color measurements for five of the factory conversions. It shows that color rises during the reaction, indicating that degradation occurs. As no significant change in dextrose equivalent, D-glucose–fructose, or other equilibrium products is apparent, color formation probably involves only a minute amount of substrate.

Hydrolyses 7, 8, 9, and 10 (Table I) summarize those made in laboratory equipment. The glucose-fructose results of the tantalum bomb reactions in which no condensation occurred compare very well with Figure 3 at 17.4% dry substance starch; the oligosaccharides are somewhat higher. Results of the laboratory converter reversion of D-glucose, in which steam condensation occurred, fall on Figure 3 at about a 14.0% dry substance level at the locus expected. Essentially the same results were obtained with laboratory as with factory equipment.

Acknowledgment

The authors thank James E. Turnbull and others of the Sugar Research Group, Clinton Foods Inc., for technical assistance; R. J. Dimler, Allene Jeanes, and C. E. Rist, Northern Utilization Research Branch, U. S. Department of Agriculture, and Alva Thompson, Ohio State University, for authentic carbohydrate samples; S. M. Cantor, Sidney M. Cantor Associates, Karl Paul Link, University of Wisconsin, and especially Geo. T. Peckham, Jr., Clinton Foods Inc., for assistance and criticism in the preparation of this paper.

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Received for review July 20, 1955. Accepted December 21, 1955. Division of Carbohydrate Chemistry, 126th Meeting, ACS, New York, N. Y., September 1954.