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# Changes in Composition of Dilute Buffered Carbohydrate Solutions Produced by Boiling.

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Because it seemed probable that variations in reaction had affected the results obtained when dilute aqueous solutions of carbohydrates were boiled,[1] the investigation was extended to include buffered solutions. Phosphate mixtures were chosen because we wished to study solutions at approximately neutral reactions. Since it seems possible that phosphate may have some specific effect upon the transformation of aldoses into ketoses[2] experiments with acetate buffer mixtures were also carried out. The effect of oxygen and nitrogen upon the reaction and the fermentability of the compounds in the boiled solutions were also investigated. The technique is described in the preceding paper.

In Table I are given the results of experiments in which the phosphate content of dilute glucose solutions was kept constant and the degree of acidity varied. It is evident that both the speed of formation of "fructose" and of decrease in the reducing power varied with the reaction: they were highest in the most alkaline and lowest in the slightly acid ones. It is worth noting that a measurable formation of "fructose" could be demonstrated when the reaction was 6.0 pH. The change at a pH of 7 resembled that found in simple aqueous solutions, for diminution in the reducing power was very slight during the first 2 hours of boiling and quite marked during the latter part of the experiment when ketose was already present in relatively high concentration. When the pH value was 7.8 a marked ketose formation accompanied the rapid destruction of sugar.

In Table II are presented the results obtained when mixtures of sodium acetate and acetic acid giving pH values over the range shown in Table I were used. The difference between the 2 sets of results was slight and probably not significant. As far as can be told neither salt had a specific effect upon the formation of ketose, but at

[1] Garbutt, H. R., and Hubbard, R. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 270.

[2] Smith, M. L., *Biochem. J.*, 1932, **26**, 1467.

TABLE I.*

Effect of Boiling 0.02 % Glucose in M/15 Aqueous Phosphate Solutions.

| Time of boiling hr. | — Buffered to 5.0 pH[1] — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 6.0 pH — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 7.0 pH — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 7.8 pH — Glucose mg./100 cc. | Fructose mg./100 cc. |
|---|---|---|---|---|---|---|---|---|
| 0 | (20)[2] | 0.25 | 20 | 0.2 | 20 | 0.2 | 20 | 0.2 |
| 2 | — | 0.25 | 20 | 0.95 | 19.8 | 4.63 | 14.7 | 6.76 |
| 4 | — | 0.25 | 19.6 | 1.67 | 18.4 | 5.8 | 9.75 | 3.67 |
| 6 | — | 0.25 | 19.6 | 2.22 | 17.5 | 5.7 | 6.9 | 2.22[3] |

* Under "glucose" is given the reducing power in terms of glucose, and under "fructose" a quantitative measure of the resorcinol reaction in terms of fructose.

[1] Approximate reading; sodium di-hydrogen phosphate alone present; reaction remained unchanged throughout the experiment.

[2] High acidity of the solution prevented a quantitative determination.

[3] The reaction of this specimen, as determined at room temperature, was 7.6 pH. The reaction of no other specimen differed from those indicated in the column headings by as much as 0.1 pH when so determined.

TABLE II.

Effect of Boiling 0.02 % Glucose in 0.2 N Aqueous Acetate Solutions.

| Time of boiling hr. | —— 6 cc. 0.2 N acetic acid —— Glucose mg./100 cc. | Fructose mg./100 cc. | pH | —— 0.2 cc. 0.2 N acetic acid —— Glucose mg./100 cc. | Fructose mg./100 cc. | pH | ——— No acetic acid ——— Glucose mg./100 cc. | Fructose mg./100 cc. | pH |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 20 | 0.2 | 5.8 | 20 | 0.2 | 7.0 | 20 | 0.2 | 7.8 |
| 2 | 20 | 0.97 | 5.8 | 20 | 4.67 | 7.0 | 15.3 | 4.63 | 7.3 |
| 4 | 20 | 1.96 | 5.8 | 17.4 | 5.88 | 7.0 | 11.8 | 4.00 | 7.1 |
| 6 | 19.8 | 2.80 | 5.8 | 15.7 | 5.70 | 7.0 | 9.5 | 3.64 | 7.2 |

All reaction values were determined at room temperature.

The amount of acetic acid in 100 cc. of each buffer mixture used is shown in the appropriate column heading.

a pH of 7 the destruction of glucose was perhaps a little more rapid in the acetate than in the phosphate solution.

In Table III results with sugars other than glucose are given. When an allowance is made for the difference in reducing power shown by the 2 sugars, figures given by mannose were practically identical with those obtained in the study of glucose. Galactose also showed a change of a similar type, but the rate of formation of ketose was apparently less rapid than that found with the other aldoses. Little or no emphasis can be placed upon this difference. The sugar was not fructose, for it was not fermentable, and data upon the relative intensity of the resorcinol reaction when various ketoses are treated by Roe's technique are not available. The sugar produced was probably tagatose.[3]

The results in Table I showed that rather slight variations in acidity had a marked effect upon the production of a ketose from glucose. The results obtained in these buffered solutions should therefore furnish fairly satisfactory material for comparing changes in glucose and fructose with each other. The rates of destruction, shown by loss in reducing power, will be discussed first. It is evident that, as in the experiments on water solutions, destruction did not begin in the solutions of the aldoses (glucose and mannose) until the 3rd or 4th hour of the experiment when ketose was already present in fairly high concentration. Fructose, however, showed destruction from the beginning of the experiment. These results suggest that glucose was first converted into a ketose and then this ketose was destroyed.

Formation of a reducing, fermentable substance from fructose is quite clearly shown by a comparison of the reducing and resorcinol reactions found after boiling. The change into an aldose was much more marked than that found in the water solutions. In the phosphate buffer giving a pH of 7 the composition of the mixtures produced by boiling fructose, glucose, and mannose for 6 hours were quite similar. Thirty-six per cent of the total reducing substances derived from fructose were still in the ketose form at that time, while glucose and mannose gave mixtures containing respectively 33 and 34% of the "total sugar" in the form of "fructose". Since 2 separate factors—the change in the sugar and the destruction of reducing substances—have cooperated in producing these results the authors do not feel justified in placing great emphasis on the finding.

[3] Armstrong, E. F., The Simple Carbohydrates and the Glucosides, third edition, London, 1913, p. 47.

TABLE III.
Dilute Solutions of Various Sugars Boiled in Aqueous M/15 Phosphate to Give pH of 7.

| Time of boiling hr. | 0.02% fructose | | | 0.02% mannose | | | 0.02% galactose | | |
|---|---|---|---|---|---|---|---|---|---|
| | Glucose mg./100 cc. | Fructose mg./100 cc. | pH | Glucose mg./100 cc. | Fructose mg./100 cc. | pH | Glucose mg./100 cc. | Fructose mg./100 cc. | pH |
| 0 | 20.6 | 18.5 | 7.0 | 18.8 | 0.2 | 7.0 | 20 | 0.1 | 7.0 |
| 2 | 17.0 | 10.6 | 7.0 | 18.7 | 4.65 | 7.0 | 19.5 | 3.16 | 7.0 |
| 4 | 15.6 | 6.68 | 7.0 | 18.3 | 5.48 | 7.0 | 19.0 | 3.72 | 7.0 |
| 6 | 13.3 | 4.88 | 7.0 | 16.7 | 5.71 | 7.0 | 16.9 | 3.72 | 7.0 |

Reaction values were determined at room temperature.

TABLE IV.
Dilute Sugar Solutions in Phosphate Boiled in the Presence of Oxygen and Nitrogen. 0.02 % Sugar in M/15 Phosphate to Give a pH Value of 7.0 Studied.

| Time of boiling hr. | Experiments with oxygen | | | | Experiments with nitrogen | | | |
|---|---|---|---|---|---|---|---|---|
| | Glucose used | | Fructose used | | Glucose used | | Fructose used | |
| | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. |
| 0 | 20 | 0.15 | 20 | 20 | 20.5 | 0.15 | 21.0 | 20.2 |
| 2 | 17.9 | 3.64 | 12.1 | 7.61 | 20.5 | 3.36 | 19.6 | 12.3 |
| 4 | 12.7 | 2.72 | 6.65 | 3.28 | 20.3 | 4.9 | 17.9 | 10.3 |
| 6 | 7.6 | 1.6 | 4.83 | 1.2 | 19.3 | 5.8 | 16.5 | 8.0 |

The reaction of each specimen was found to be 7.0 pH when determined at room temperature.

The results given in Table IV are those obtained when gases were bubbled through boiling solutions containing phosphate to give a neutral reaction. They should be compared, not only with others in this article, but also with those in Table II of the preceding one. In the nitrogen experiment the rate of formation of ketose from glucose was approximately the same as when the gas was not used. It is evident, however, that the rate of decrease of the reducing power shown by both sugars was less when this gas was used than in the control experiment, and that the amount of ketose still present in the fructose solution after 6 hours boiling was approximately twice as great in the former as in the latter. The authors interpret these figures as supporting their thesis that loss in reducing power represents destruction of sugar, or, more properly, conversion of sugar into some non-reducing substance, and that this change is largely at the expense of the ketose. The complicated nature of the reactions in these solutions of course makes it impossible to decide whether this represents the only, or even the main, cause of the loss of reducing power found.

A comparison of the composition of the mixture obtained by boiling these 2 sugars for 6 hours is interesting. Fructose still contained 48% of the total reducing substances in the form of a ketose at that time, while glucose, after similar treatment, gave a mixture with only 30% in that form. It is evident that the agreement shown by the figures discussed above was markedly influenced by differences in the rates at which the 2 sugars are destroyed. The significance of the figures as an expression of an equilibrated mixture seems therefore questionable.

The effect of running oxygen through the boiling mixture was striking. The reducing power of both the glucose and the fructose solutions decreased rapidly; fructose showed the greater change. Since such a result was not found when a water solution containing no phosphate buffer was treated in a similar way it seemed possible that the phosphate had had a specific effect upon the change. The probability that such a specific effect existed was strengthened by an experiment in which glucose was buffered to a pH of 7, with an appropriate acetate mixture and boiled in the presence of oxygen gas. The results were almost identical with those obtained in a similar solution not treated with gas. (Table II.) The composition of the mixture at the end of 6 hours was: "glucose" 15.7 mg./100 cc.; "fructose" 5.2 mg./100 cc.; reaction 7.0 pH.

The product obtained by boiling these dilute sugar solutions in the neutral phosphate buffer mixture was fermented by treating 5 cc.

of the solution with one cc. of packed, washed yeast cells for 10 minutes. The initial solutions of glucose, fructose and mannose showed no reducing or resorcinol reaction after one treatment of this kind; the boiled solutions, however, regularly showed both reactions after repeated treatments, suggesting that some non-fermentable sugar (glutose?[4]) was present. The average values were: From glucose: 1.5 mg. "glucose" per 100 cc.; 0.59 mg. "fructose" per 100 cc. From fructose: 2.2 mg. "glucose" per 100 cc.; 1.2 mg. "fructose" per 100 cc. From mannose: 1.5 mg. "glucose" per 100 cc.; 0.7 mg. "fructose" per 100 cc. Similar analysis of boiled 0.1% solutions after prolonged incubation with yeast gave similar results; the actual amounts of "glutose" were larger, but the relationship between the amounts obtained from glucose and levulose, and the relative intensities of the reducing and resorcinol reactions were similar.

When dilute solutions of hexoses containing buffers to give reactions which were neutral, or which varied only slightly from a pH of 7 were boiled the following effects were observed: a molecular rearrangement similar to that shown by alkaline solutions, with a formation of fructose from glucose and mannose, and of a mixture of aldoses from fructose took place; a formation of fructose from glucose was demonstrated at a pH of 6; simultaneously with these changes there occurred a destruction of sugar, which was shown by a decrease in the total reducing power; this destruction of sugar was most marked in the slightly alkaline solutions, and seemed to take place largely at the expense of the ketose present; the change of one form of sugar into another was independent of the nature of the salt mixture used in buffering the solution; phosphate increased markedly the rate of destruction of sugars by oxygen; a small amount of a non-fermentable sugar was formed in solutions of pH 7.

[4] Benedict, E. M., Dakin, H. D., and West R., *J. Biol. Chem.*, 1926, **68**, 1.