of cane sugar in passing from the unripe to the ripe condition and its subsequent inversion in passing from the ripe to the overripe condition. In the unripe stage we have less than 0.5% of cane sugar present, whereas in the ripe stage we have as much as 3.61% of cane sugar. The cane sugar is quickly inverted and we have only a little more than 0.5% of cane sugar in the overripe stage.

From the results obtained in this investigation the following conclusions may be drawn:

First, that there is a gradual diminution in the acidity of this fruit during the ripening period and at the same time there is a more pronounced increase in the amount of reducing sugar formed.

Second, the greatest increase in total sugars occurred in passing from the unripe to the ripe condition.

Third, that cane sugar plays a very important part in the ripening of this fruit, and the idea is suggested that a fruit is just ripe when it contains the maximum amount of cane sugar.

Fourth, that this fruit contains the enzyme invertase, which is most active in passing from the ripe to the overripe stage.

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[CONTRIBUTION FROM THE ORGANIC LABORATORY, COLUMBIA UNIVERSITY, AND THE HARRIMAN RESEARCH LABORATORY. NO. 260.]

THE INFLUENCE OF CERTAIN SUBSTANCES ON THE ACTIVITY OF INVERTASE.

BY EDWARD G. GRIFFIN AND J. M. NELSON.

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Effect of Glass Beads.—Beard and Cramer[1] have shown that glass beads have an inhibiting influence on the activity of lipase, diastase and invertase, and claim that this is due to the alteration in the concentrations, produced by altering the surface energy at the larger surface of the glass beads. They found that the effect increased with the time and also with the temperature, and that a part but not all of the activity was regained when the beads were removed.

As glass is appreciably soluble in water and gives to it an alkaline reaction, it seems probable that these results were due to a change in the hydrogen-ion concentration rather than to the surface of the beads. The following experiments serve to confirm this view:

Portions of 50 cc. of a 10% cane-sugar solution with 1 cc. of invertase solution, were placed in Non-sol glass bottles with varying amounts of glass beads, and the inversion allowed to take place.

Grams of glass beads	0.0	25	50	100
Conc. of H^+	$10^{-5.9}$	$10^{-6.5}$	$10^{-7.8}$	$10^{-8.5}$
Change in rotation in 24 hrs.	0.34°	0.11°	0.06°	0.02°

[1] *Proc. Roy. Soc.*, (*B*) **88**, 575 (9115).

The hydrogen-ion concentration was determined by suitable indicators. It will be noticed that the hydrogen-ion concentration decreases as the amount of the glass beads increases. As Sörensen,[1] Fales and Nelson[2] and others have shown, that the optimum for invertase for these conditions is $H^+ = 10^{-4.4}$, and that the activity is very small at $H^+ = 10^{-8}$, it seems clear that the alkalinity from the glass beads is the cause of the effect produced.

The following results with the solutions containing 50 cc. of a 20% cane-sugar solution, 10 cc. of invertase solution and 50 cc. of one of Sörensen's "buffer solutions" confirm this view:

TABLE I.

	Grams of glass beads.	Temperature.	$p_H{}^+$.	Changes in rotation. 24 hrs.	48 hrs.	72 hrs.
A[3]	100	37	4.9	1.82°	3.91°	5.03°
C[3]	100	37	4.9	1.80°	3.88°	5.10°
B[4]	...	37	6.7	1.23°	2.32°	3.14°
D[4]	...	37	6.7	1.22°	2.31°	3.25°
E[4]	100	20	6.0	0.98°	...	...
F[4]	...	20	6.0	1.07°	...	...

In E and F a weaker invertase solution was used.

$p_H{}^+$ is Sörensen's symbol for expressing the concentration of the hydrogen ion. The values are the negative exponents to which the number 10 must be raised in order to equal the given concentration. Thus $p_H{}^+ = 4.5$ means that the hydrogen-ion concentration is equal to $10^{-4.5} = 0.00003$ mols hydrogen ion per liter.

These experiments show that the glass beads produce no effect either at 20° or 37° when the hydrogen-ion concentration is kept constant by means of the "buffer solutions."

After the work described was completed, the attention of the authors was called to a statement of Armstrong and Armstrong[5] in which they suggest that the effect of the glass beads is probably due to the solubility of the glass. Since they made no measurements and Beard and Cramer still insist on their original conclusions,[6] it seems worth while to publish these results.

Effect of Serum and Egg Albumin on the Activity.—Eriksson[7] found that serum neutralized with very dilute hydrochloric acid inhibited the activity of invertase, and the extent of this effect was dependent on the order of mixing of the substrate, enzyme and inhibitor, and also on the

[1] *Biochem. Zeit.*, 21, 131 (1909).
[2] THIS JOURNAL, 37, 2786 (1915).
[3] Buffer solution, sodium citrate and hydrochloric acid.
[4] Buffer solution, primary and secondary phosphate mixture.
[5] *Nature*, 95, 425 (1915).
[6] *Ibid.*, 95, 561 (1915).
[7] *Z. physiol. Chem.*, 72, 324 (1911).

length of time of contact of the enzyme and inhibitor before addition to the substrate. The addition of acid lowered this inhibiting effect. Hedin[1] noted that serum and egg albumin had a similar effect on the activity of rennet. Both Eriksson and Hedin attribute this retarding influence to the enzyme combining with the inhibitor in an "irreversible or slightly reversible" way, thereby causing the enzyme to become inactive.

A series of experiments, similar to those of Eriksson, was undertaken in which the particular hydrogen-ion concentration of each solution was determined, and the results obtained showed that when the latter was kept constant, no retardation occurred.

TABLE II.

	Solutions used.					
	Cc. 10% cane-sugar soln.	Cc. invertase soln.	Cc. serum soln.	Cc. water.	Cc. buffer soln.	Cc. 0.1 *M* HCl soln.
A[2]	80	5	10	5	..	...
B[3]	80	5	10	5	..	...
C	80	5	..	..	15[4]	...
D	80	5	..	15	..	...
E	50	2	10[6]	3.5	..	1.5
F	50	2	..	5	10[5]	...
G	50	2	..	15	..	...
H	50	2	10[6]	5	..	...
J[2]	80	5	5	10	..	...
K[3]	80	5	5	10	..	...
L	80	5	..	..	15[4]	...

TABLE III.

Solution.	A.	B.	C.	D.	J.	K.	L.	E.	F.	G.	H.
p_H+	8.2	8.2	8.2	5.9	8.1	8.1	8.1	4.3	4.3	5.9	8.1
Change in rotation in 24 hrs.	1.37°	1.38°	1.36°	2.19°	1.56°	1.48°	1.47°	1.74°	1.77°	1.35°	0.53°

In comparing the behavior of Solutions A and B with that of C in Table III, it becomes evident that the serum has no inhibiting influence on the rate of inversion. The values for D show that the hydrogen-ion concentration in the control solution must be the same as that of the serum solutions, if comparable results are to be obtained. The apparent retardation observed in contrasting the values of A and B with that of D is therefore not due to any influence of the serum except in so far as the serum causes the solution to become more alkaline. Eriksson was aware of the tendency of the serum to cause the solution to become alkaline, and that a change

[1] *Z. physiol. Chem.*, 60, 85, 364 (1909).

[2] The invertase and serum were mixed and allowed to stand for one hour at 37° before adding the rest of the solution.

[3] All parts of the solution were mixed at once.

[4] Phosphate mixture.

[5] Citrate and hydrochloric acid.

[6] A 25% aqueous serum solution.

in the acid or alkaline condition of the solution influenced the activity of the enzyme. In order to avoid this source of error in his measurements of the retardation, he neutralized the serum with hydrochloric acid, but apparently he did not neutralize to any particular hydrogen-ion concentration, nor did he bring the hydrogen-ion concentration of the control solution to the same value as that of the serum solution. He also states that as the serum is neutralized, the inhibiting influence of the serum diminishes, but is not completely destroyed as observed by Hedin in the case of some other enzymes. The values from Solutions E, F and H show that when the serum is neutralized with hydrochloric acid as in the case of E, the lowering of the inhibiting effect is only a question of the relative hydrogen-ion concentrations. The value from G shows further that the serum in E has an accelerating effect instead of a retarding one, due to the value of p_H+ being 4.3, which is closer to the optimum hydrogen-ion concentration for invertase than the value of p_H+ in G, which is 5.9. The values obtained from Solutions A and B, where the order of mixing of the serum, cane sugar, and invertase was varied, are the same, therefore nullifying this contention of Eriksson. Upon comparing the values of J, K and L with those of A and B, it is to be noted that the amount of serum used has no effect. Similar results were obtained when egg albumin was used in place of serum, as will be noticed from the results obtained in the series of experiments shown in Table IV.

TABLE IV.

The solutions used all contained besides 80 cc. of 10% cane-sugar solution and 5 cc. of invertase solution, the following amounts of:

	Cc. egg albumin.	Cc. water.	Cc. buffer soln.	Cc. 0.1 *M* HCl.		Cc. egg albumin.	Cc. water.	Cc. buffer soln.	Cc. 0.1 *M* HCl.
A	1	14	..	..	H	5	7	..	3
B	5	10	..	..	I	5	4	..	6
C	15	..	..	..	J	5	2	..	8
D	..	..	15[1]	..	K	..	..	15[2]	..
E	..	..	15[1]	..	L	..	..	15[3]	..
F	..	..	15[1]	..	M	..	..	15[3]	..
G	..	15	..	..					

TABLE V.

Solution.	A.	D.	B.	E.	C.	F.	G.	H.	K.	I.	L.	J.	M.
p_H+	9.0	9.0	9.3	9.3	9.5	9.5	5.8	6.5	6.5	4.3	4.3	3.7	3.7
Change in rotation, 3 hrs.	0.61°	0.55°	0.36°	0.37°	0.01°	0.00°	2.35°	....	....	....	....	....	....
Change in rotation, 6 hrs.	....	....	....	....	....	....	....	4.58°	4.62°	10.3°	10.3°	9.67°	9.69°

The amount of inversion in Solutions A, B, C and G, shown in Table V, indicate an apparent retardation of the activity of the invertase by the

[1] Sodium borate and sodium hydroxide.

[2] Phosphate mixture.

[3] Sodium citrate and hydrochloric acid.

increasing amounts of egg albumin in the solution. The results from D, E and F, however, show that this retardation is due to the change in hydrogen-ion concentration of the solutions caused by the egg albumin, and that the presence of the latter has nothing to do with the rate of inversion. The results from H, I, J, K, L and M show that neutralizing the egg albumin with dilute hydrochloric acid has no effect outside of changing the hydrogen-ion concentration.

In order to see whether any different effect occurred when the egg albumin was allowed to remain in contact with the invertase for some time before it was added to the substrate as claimed by Hedin in the case of rennet, the following experiments were undertaken.

Three solutions were used for this purpose:

A. 15 cc. of a solution (prepared by beating together 30 cc. of egg albumin and 33 cc. of a 0.1 *M* solution of hydrochloric acid and then making up to 100 cc. with water) and 5 cc. of an invertase solution were allowed to stand for 1 hour and then added to 80 cc. of a 10% cane-sugar solution.

B. The same amounts of egg albumin, invertase, and cane-sugar solutions, all were mixed at once.

C. 15 cc. of a solution containing enough of a mixture of sodium citrate and hydrochloric acid to give the same hydrogen-ion concentration as that of A and B, were added to 80 cc. of the cane-sugar solution and 5 cc. invertase.

TABLE VI.

Solution.	A.	B.	C.
p_H^+	4.5	4.5	4.5
Change in rotation in 2.5 hrs.	10.38°	10.34°	10.33°

The results show that in the case of invertase at least, it makes no difference whether or not the enzyme and the egg albumin are allowed to remain in contact for some time before being added to the substrate.

Effect of Charcoal on the Activity of Invertase.—Eriksson[1] also found in studying the influence of animal charcoal on the activity of invertase that it too, like serum and egg albumin, had an inhibiting effect, which was greater when the charcoal and invertase were mixed and allowed to stand in contact for some time before being added to the cane-sugar solution, than when all three, enzyme, inhibitor, and substrate, were mixed at once.

The experiments of Eriksson were repeated and the results obtained (Table VII) showed that in this case also it was only a question of hydrogen-ion concentration.

The values of p_H^+ for Solutions A and C, the latter especially, when compared with that of Solution E, show that the presence of charcoal does effect the hydrogen-ion concentration of the solution. When buffer was added to Solution E as in Solutions B and D, so that p_H^+ was of

[1] *Loc. cit.*

the same value as that of Solutions A and C, respectively, then the activity of the invertase was the same as can be seen by comparing the values of A with B, and C with D. The inhibiting effect of charcoal must, therefore, be attributed to a change in the hydrogen-ion concentration. The values from Solutions F, G, H, and I and their controls show that the same holds true for charcoal at different concentrations of hydrogen ion as long as $p_H{}^+$ is of the same value as that of the control solution. Several different charcoals were used and the results obtained, all agreed with those found in the case of the above solutions and therefore have been omitted in the table.

TABLE VII.

The solutions used contained, besides 80 cc. of 10% cane-sugar solution and 10 cc. of an invertase solution, also the following:

	Gram charcoal.	Cc. buffer solution.	Cc. water.
A[3]	0.2[1]	..	10
B	...	10	..
C[3]	0.2[2]	..	10
D	...	10	..
E	...	..	10
F[4]	0.2[1]	10	..
G[4]	0.2[1]	10	..
H[4]	0.2[1]	10	..
I[4]	0.2[1]	10	..
J	...	10	..
K	...	10	..
L	...	10	..
M	...	10	..

J, K, L and M were controls for F, G, H and I.

Solutions F–M, inclusive, contained stronger invertase.

TABLE VIII.

Solution.	A.	B.	C.	D.	E.	F.	J.	G.	K.	H.	L.	I.	M.
$p_H{}^+$....	6.0	6.0	6.7	6.7	5.9	3.1	3.1	4.1	4.1	4.8	4.8	7.1	7.1
Change in rotation, in 5 hrs.	2.50°	2.48°	2.08°	2.11°	2.54°	9.54°	9.55°	10.47°	10.48°	10.57°	10.56°	6.97°	6.95°

In measuring the amount of inversion of the cane sugar in the above experiments, the bottle containing the reaction mixtures was thoroughly shaken and a sample removed, treated with sodium carbonate solution, and then read in the polariscope. The amount of cane sugar and invert sugar held back by this small amount of charcoal was not sufficient to make any appreciable difference in the readings. When larger amounts of charcoal were used, the adsorption of the sugar became noticeable and therefore the effect could not be measured satisfactorily.

[1] Finely powdered animal charcoal of a very good adsorbing power.

[2] Finely powdered animal charcoal ordinarily used in the organic laboratory.

[3] The charcoal and invertase were mixed first and allowed to stand for 1 hour before adding the rest of the solution.

[4] All parts of the solution were mixed at once.

The following experiments (Table IX) will illustrate this point. Three mixtures of charcoal, cane sugar and water were prepared by allowing 2, 5 and 10 g. of finely powdered animal charcoal to stand in contact with 100 cc. of cane-sugar solution for 18 hours with occasional shaking. Sixty cc. portions of each of these mixtures were added to solutions consisting of 10 cc. of primary potassium phosphate and 10 cc. of invertase solution and marked A, B and C, corresponding respectively to the 2, 5 and 10 g. of charcoal.

TABLE IX.

Solution.	p_H^+.	Change in rotation after 0 hours.	Change in rotation after 24 hours.
A.....	4.9	0.05°	1.63°
B.....	5.0	0.10°	1.40°
C.....	5.0	0.24°	1.34°

The changes in rotation indicated in the 0 hours column are very likely due to the removal of some of the cane sugar by the charcoal during the 18 hours in which they remained in contact before the invertase was added. The values in the 24 hours column seem to indicate that in this case also some sugar might have been taken up by the charcoal, otherwise they ought to have been practically the same. No evidence for the presence of invert sugar in any of the above solutions after standing for 18 hours and before adding the invertase, could be detected by means of an alkaline copper solution.

Michaelis and Ehrenreich[1] found that invertase was adsorbed by relatively large amounts of charcoal, and although the filtrate from the charcoal and invertase solution showed no presence of invertase, the unfiltered mixture was still active.

Results obtained in this laboratory confirm those of Michaelis and Ehrenreich. It was found also that if small enough quantities of charcoal were employed, so that the effect of the charcoal on the concentration of the sugar was very small, thereby permitting the measurement of the amount of inversion, the activity of the invertase was unaffected by the charcoal.

The Effect of Aluminium Hydroxide on the Activity.—Michaelis and Ehrenreich also found invertase to be adsorbed, at least partially, by gelatinous aluminium hydroxide. Welker and Marshall[2] have made similar observations in the case of other enzymes. They failed, however, to indicate the particular hydrogen-ion concentration of the solutions employed. The question therefore arises as to whether the hydrogen-ion concentration of their solutions might not have been responsible for some of this change in activity. In order to settle this point, their work was

[1] *Biochem. Z.*, 10, 294 (1908).

[2] THIS JOURNAL, 35, 822 (1913).

repeated. It was found that aluminium hydroxide does remove invertase completely from its solution. If, however, the invertase is allowed to act on cane sugar, in the presence of aluminium hydroxide, just as in the case of small amounts of charcoal, it apparently has no effect on the activity of the enzyme, as will be observed from the following set of experiments:

Twenty cc. of freshly precipitated aluminium hydroxide, washed free from chlorides and ammonia and containing aluminium equivalent to 0.083 g. of aluminium oxide, were mixed with 20 cc. of invertase solution and filtered.

A. Twenty cc. of this filtrate were added to 25 cc. of a 10% cane-sugar solution.

B. This solution consisted of 10 cc. of invertase solution, 10 cc. of the above aluminium hydroxide and 25 cc. of the cane-sugar solution.

C. A control solution consisting of 10 cc. of a solution of phosphates necessary to give the proper hydrogen-ion concentration, 10 cc. of invertase solution and 25 cc. of cane-sugar solution.

TABLE X.

Solution.	p_H^+.	Change in rotation after 1 hour.	3 hours.	4 hours.
A	6.3	0.00°	0.01°	0.00°
B	6.3	1.08°	2.43°	2.83°
C	6.3	1.07°	2.40°	2.80°

Experimental Part.

The hydrogen-ion concentrations were determined by the electromotive force method described by Fales and Nelson,[1] except where otherwise stated. In case there was any possibility of a change in the hydrogen-ion concentration during the time of inversion, the values of p_H^+ were determined at the beginning and end of the inversion.

The indicator method outlined by Sörensen proved inapplicable in the case of solutions containing colloids as serum and albumin. The values for p_H^+ obtained by means of indicators are compared with those from the electromotive-force method in Table XI.

TABLE XI.

Solution	Value of p_H^+ by the two methods. α-Naphthylamino-azo-benzene sulphonate.	Methyl orange.	E. M. F.
and 3.5 cc. acid	4.7	3.4	3.0
and 2.5 cc. acid	5.1	3.8	3.4
and 1.8 cc. acid	5.3	4.4	4.0
Control solution			
and 0.8 cc. acid	...	3.0	3.0
and 0.4 cc. acid	...	3.4	3.4
and 0.1 cc. acid	4.0	4.0	4.0

[1] *Loc. cit.*

Five cc. containing varying numbers of cc. of 0.1 *M* hydrochloric acid and the rest water, were added to a solution consisting of 10 cc. of a 25% serum, and 50 cc. of a 10% cane-sugar solution. The control solution differed from this by having the same volume of water in place of the 10 cc. of serum solution.

All the inversions took place in Non-sol glass bottles at 37°, unless otherwise stated. The solutions were always preserved with toluene.

The invertase was obtained from yeast by the method of Nelson and Born.[1] The enzyme and substrate solutions were always warmed to 37° before mixing. The enzyme activity was stopped, and mutarotation effect due to the invert sugar was overcome by means of sodium carbonate as recommended by Hudson,[2] except in the case of solutions containing serum or egg albumin. When the latter were present, it was not necessary to add the sodium carbonate, since the reagent added to remove the protein seemed to have the same effect.

The serum and egg albumin in most cases were removed from the solutions before the latter were examined in the polariscope, by a slight modification of the method suggested by Kumagai.[3] Ten cc. of the solutions were introduced into a 150 cc. Erlenmeyer flask containing 18 cc. of a 10% sodium acetate solution. Two cc. of a ferric chloride solution were then added and the mixture heated on the water bath for six minutes and filtered hot. The clear filtrate was allowed to cool and its rotation determined.

The serum used in these experiments was prepared by centrifuging fresh, defibrinated sheep's blood.

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NEW BOOKS.

A Text-book of Inorganic Chemistry. Edited by J. NEWTON FRIEND. Vol. VIII. **The Halogens and Their Allies.** By GEOFFREY MARTIN AND E. A. DANCASTER. Pp. xviii + 337. Philadelphia: Lippincott, 1915. Price, $3.00.

In order of publication, this is the second volume of the nine into which this text-book is divided (for review of Vol. I, see THIS JOURNAL, **37**, 1641 (1915)). In many ways, the high standard of the first volume is maintained. After a concise introduction, the halogens and manganese, and their compounds with the nonmetallic elements, are described. Numerous references to the literature are given. Manufacturing processes are discussed in detail, with figures of the plant used. The familiar lecture experiments are also fully described, and are illustrated by cuts. Numerical data are abundant and appear as a rule to be judiciously se-

[1] THIS JOURNAL, **36**, 393 (1914).
[2] *Ibid.*, **30**, 1564 (1908).
[3] *Biochem. Z.*, **57**, 380 (1913).