Subs. (vacuum-dried), 0.4972, 0.4972: 53.45, 53.38 cc. of 0.1 N H_2SO_4. Calc. for $C_6H_2O_2(NOH)_2.H_2O$: N, 15.06. Found: 15.06, 15.04.

Subs. (dried at 105°), 0.4304, 0.5958: 50.84, 70.28 cc. of 0.1 N H_2SO_4. Calc. for $C_6H_2O_2(NOH)_2$: N, 16.67. Found: 16.55, 16.53.

Subs. (vacuum-dried), 0.2054, 0.2194: CO_2, 0.2933, 0.3118; H_2O, 0.0622, 0.0675. Calc. for $C_6H_2O_2(NOH)_2.H_2O$: C, 38.71; H, 3.25. Found: C, 38.95, 38.77; H, 3.40, 3.44.

The decomposition point of the dinitroso-resorcinol was found to be 162–163°, and not 115°.

In addition to the solubility in the various solvents mentioned by Fitz, it was found that dinitroso-resorcinol is insoluble in toluene, carbon tetrachloride or petroleum ether in the cold and very slightly soluble in these solvents at the boiling point. It is very slightly soluble in chloroform. An aqueous solution of the above dinitroso-resorcinol also gave the characteristic green color with ferric chloride solution, described by Fitz.

Summary

1. A method has been given for the preparation, purification and crystallization of dinitroso-resorcinol.

2. Dinitroso-resorcinol, when dried to constant weight in a vacuum desiccator over sulfuric acid, contains 1 molecule of water of crystallization and has the composition indicated by the formula $C_6H_2O_2(NOH)_2.H_2O$.

3. Pure crystalline dinitroso-resorcinol decomposes at 162–163° instead of at 115° as is almost always stated.

4. The action of several solvents upon dinitroso-resorcinol has been studied.

5. The crystal form and characteristics of dinitroso-resorcinol have been determined.

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[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, THE ROOSEVELT HOSPITAL]

STUDIES ON ENZYME ACTION. XXIII. THE SPONTANEOUS INCREASE IN SUCRASE ACTIVITY OF BANANA EXTRACTS

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Introduction

The enzymic hydrolysis of sucrose has been investigated to a greater extent probably than any other enzyme action. Reference may be made especially to the pioneering work of O'Sullivan and Tompson, and to the more recent extensive studies of Sörensen, Euler, Hudson, Michaelis, Nelson, and Willstätter, and their co-workers. These studies were concerned practically entirely with the sucrase obtained from yeast, sucrase from other sources being considered only incidentally. It seemed, therefore, to be of some interest to study more intensively the sucrase from a

source other than yeast. Several papers[1] dealing with the properties of banana sucrase have been published pursuant to this plan. In studying this problem further it was found, in following the sucrase actions of banana extracts, that under certain conditions these actions showed apparently spontaneous increases of considerable magnitude. The conditions under which these increases occurred will be described in this paper.

Experimental Methods and Results

The experimental work will be described in the following order: (1) methods used; (2) increases in activity observed with various extracts; (3) results with the different conditions successively controlled.

The extracts were prepared as described in detail in previous papers.[1] The ground pulp of bananas was extracted with solutions of different salts, the ratio of grams of pulp to cubic centimeters of extracting liquid being 2:1. The mixtures were filtered through paper, and the filtrates used for the various experiments. Because of the slow filtration, the preliminary treatment usually required from 4 to 24 hours at room temperature, depending upon the quantity of extract desired. Unless stated otherwise, toluene was added to the mixtures during the filtrations.

The sucrase actions were measured by incubating the enzyme preparations suitably diluted with sucrose solution for periods ranging from 1 to 4 hours at $37.5° \pm 0.05°$, and determining the reducing substances with Fehling solution.[2] The time for any one series was constant and the results therefore comparable, suitable corrections for blanks being introduced in every case. The results are given as milligrams of cuprous oxide obtained from the reducing substances formed during the incubation period by 1 cc. of dialyzed extract or calculated to the common basis of 1 cc. of undialyzed extract.

In Table I are shown the results obtained when banana pulp extracts, given with different solutions and dialyzed to remove most of the salts and simple reducing substances, were allowed to stand in the ice box for different lengths of time, toluene being present throughout. Five different batches of bananas were used.

The main conclusion to be drawn from the results given in Table I is that there is a very marked increase in sucrase activity with every extract. Different salt extractions showed different increases. The water extract gave the smallest action but the largest percentage increase. The different batches of bananas showed different amounts and rates of increase with the same salt extract. At least one (B 1) appeared to have reached a maximum. In general terms, the increase ranged from 40% to 100% in 2–3 days at 5–10° as compared with the action found immediately after filtration and dialysis.

[1] Falk and McGuire, *J. Gen. Physiol.*, **3**, 595 (1921); *J. Biol. Chem.*, **54**, 655 (1922).

[2] Sherman, Kendall and Clark, THIS JOURNAL, **32**, 1083 (1910). McGuire and Falk, *J. Gen. Physiol.*, **2**, 217 (1919–1920).

TABLE I

INCREASE IN SUCRASE ACTIONS OF DIALYZED BANANA EXTRACTS AT 5–10° (TOLUENE PRESENT)

Expt.	Extracting liquid *M*	Salt	Extracts dialyzed in tap water Hrs.	Sucrase actions (calc. per cc. of undialyzed extract) of dialyzed extract after standing at 5–10° 0 Hours	24	48	72	96	120	168
A 1	Water		4	27	...	...	99	...	...	...
A 2	1.0	NaCl	4	221	...	...	356	...	...	...
A 3	0.2	$NaNO_3$	4	251	...	...	359	...	...	...
A 4	0.2	$MgSO_4$	4	251	...	...	351	...	...	...
A 5	0.2	Na_2SO_4	4	235	...	...	415	...	...	...
B 1	0.2	NaCl	4	331	341	499	...	...	441	...
C 1	0.6	Na_2SO_4	3.5	220	...	358	451	...	...	...
C 2	0.6	Na_2SO_4	3.5[a]	332	...	...	...	601	...	...
D 1	0.6	$MgSO_4$	4	322	398	...	...	...	...	550
E 1	0.2	NaCl	20	279	...	396	...	...	...	...

[a] Distilled water.

The extract blanks in the tests for sucrase actions were small in every case because dialyzed extracts were used. They showed no significant change on prolonged standing, sometimes decreasing slightly and sometimes increasing slightly, these differences probably representing experimental inaccuracies only. The undialyzed extracts were not studied because the presence of so much reducing substance in the mixtures not only gave large blanks in the sucrase tests but also, in all probability, interfered with the sucrase actions themselves, for the total reducing action on incubation of the undialyzed extract plus sucrose minus the large undialyzed extract blank was always less than the total action of the dialyzed extract similarly tested minus the small dialyzed extract blank.

The increases in the sucrase actions given in Table I occurred at low temperatures. A number of series were run at different temperatures. The results of two of these are shown in Table II.

TABLE II

INCREASE IN SUCRASE ACTIONS OF DIALYZED EXTRACTS AT DIFFERENT TEMPERATURES (TOLUENE PRESENT)

0.6 *M* Na_2SO_4 extract dialyzed 3.5 hours against tap water			0.2 *M* NaCl extract dialyzed 4 hours against tap water		
Times and temperatures of standing 5–10° Hours	37° Hours	Sucrase actions	Times of standing Hours	Sucrase action after standing at 5–10°	Sucrase action after standing at 20°
0	0	220	0	331	331
48	0	358	24	341	369
48	1/6	430	48	499	535
48	1	452	120	441	398
72	0	451	...	...	...
71	1	491	...	...	...
70	2	453	...	...	...
68	4	415	...	...	...
66	6	286	...	...	...

The interesting feature of these results is the increase in activity followed by a decrease, the changes taking place in shorter time intervals at the higher temperatures.

As it is possible that the toluene was involved in the increased sucrase actions described, a series was run at ice-box temperature comparing the actions with toluene and with chloroform as preservatives and in the absence of any preservative. The results are shown in Table III. A 0.2 *M* sodium chloride extract was filtered through paper (toluene present during this filtration); separate portions were then dialyzed for 4 hours against tap water (no preservative, toluene, or chloroform added in the different series beginning at this point), allowed to stand for the indicated lengths of time, and then tested for sucrase actions (3 hours at 37.5°, the results calculated as milligrams of cupric oxide produced per cubic centimeter of original undialyzed extract).

TABLE III

INCREASE IN SUCRASE ACTIONS OF DIALYZED BANANA EXTRACT WITH TOLUENE, CHLOROFORM, AND NO PRESERVATIVE PRESENT

	Sucrase actions after different periods of time at 5–10°			
Treatment	Hours 0	24	48	120
No preservative	331	340	496	423
Chloroform	331	355	524	393
Toluene	331	341	499	441

The results in Table III for toluene, chloroform, and no preservative are sufficiently similar to show that the increase in activity of banana extract on standing is not characteristic of the presence of toluene.

In order to account for the observed results three obvious explanations are suggested, namely, (1) new enzyme formation due to bacterial growth, (2) liberation of sucrase by destruction of banana cells and (3) change in hydrogen-ion concentration to produce more favorable conditions for enzyme action, nothing having been added to the extracts to control or fix the acidity in the experiments so far described.

With reference to the first explanation, the presence of toluene throughout eliminated bacterial growth. The absence of cells, either bacterial or from the bananas, was shown by staining the air-dried slides of the dialyzed extracts with (a) methylene blue, (b) carbo-fuchsin, (c) Bismarck brown and (d) gentian violet.[8] Also, the original filtration of the pulp mixtures through paper would be expected to remove any banana cells, and the fact that a dialyzed extract was centrifuged in a Sharples supercentrifuge to remove all suspended matter (including any cells that might be present) and the clear liquid increased in activity 40% in 6 days in the ice box, may be taken as confirmatory evidence of the absence of cells.

[8] Thanks are due Miss E. M. Fletcher of the Bacteriological Laboratory, The Roosevelt Hospital, who performed these experiments.

The results so far presented show that various extracts of banana pulp, on standing, first increased in sucrase activity and if kept a sufficient length of time later decreased, that these changes were independent of the nature of the extracting solution, water and the different salt solutions showing similar relations, that cell growth or cell functions were not concerned in the changes, and that the presence or absence of certain antiseptic substances was not involved.

In the experiments to be described, in which the possibility of a change in hydrogen-ion concentration accounting for the results was studied, the following conditions were kept constant: 0.6 *M* magnesium sulfate solution for extraction; less than 1 hour filtration; dialysis of 25cc. portions of the filtrates in collodion bags against running tap water for 2–3 hours, toluene present. Hydrogen-ion concentrations were determined with indicators and color comparison with standard solutions whenever possible.

The activities of the extracts were studied as dependent upon the following factors: (1) the hydrogen-ion concentration with hydrochloric acid or sodium hydroxide added; (2) the hydrogen-ion concentration in the presence of buffer mixtures made up of different substances and in various concentrations; and (3) the age of the extracts. The third factor is involved with one or the other of the first two in every experiment.

In an earlier paper,[1] a P_H activity curve for banana sucrase was given showing an optimum zone between P_H 3.5 and 5.0, with rapid falling off in activity in more acid and more alkaline solutions. The hydrogen-ion concentrations of the solutions were fixed by the addition of sodium hydroxide and hydrochloric acid. The optimum was very nearly at P_H 4.0, where the activity was about 6% to 7% greater than at P_H 5.0.

In the course of the work described in this paper a great number of experiments were carried out to determine the hydrogen-ion concentration of the dialyzed banana extracts which were used in determining the activities. In the enzyme action incubations, 5 cc. of such extract was mixed with 15 cc. of 20% sucrose solution and 0 to 3 cc. of water or buffer mixture. Where acid or alkali was used, this was added directly to the enzyme preparation to bring to the desired hydrogen-ion concentration and the mixture added to the sucrose solution. An aliquot portion was used for the estimation of the reducing substance, as described elsewhere. The Sörensen value of the untreated dialyzed banana extract as well as of its incubated mixtures was very nearly 5.0 with methyl red, methyl orange, bromocresol purple, and bromophenol blue as indicators. There was no apparent change in this value as determined by indicators on the aging of the juice. However, no change could be observed upon the addition of small amounts of acid to the juice until the colors indicated that a P_H of 4.5 was reached.

In place of all of the experimental results obtained, a few typical examples only will be given.

In Figs. 1 and 2 are shown the results obtained with a banana extract prepared as described in which the mixtures were brought to definite hydrogen-ion concentrations as shown by indicator colors, in one case by the addition of hydrochloric acid or sodium hydroxide, the amounts added ranging from 0.11 to 1.8 millimoles of hydrochloric acid and 0.39 to 1.5 millimoles of sodium hydroxide per liter of the mixtures tested, in the other case by the addition of 1.5 cc. of citrate buffer mixture,[4] to 20 cc. of sucrose-banana mixture. The sucrase activities were determined immediately, and also after the banana extract had been allowed to stand for various lengths of time at 5–10° and then brought to the requisite hydrogen-ion concentrations. In the plotting of the curves, the variable factors are the amounts of the actions, the hydrogen-ion concentrations, the concentrations and natures of the salts, and the ages of the extracts. In order to show a series of such tests in one plot, the ages of the extracts are plotted as abscissas and the amounts of actions as ordinates. Each series tested at a definite hydrogen-ion concentration would then be shown as 1 curve on the plot. A set of results for the different hydrogen-ion concentrations for a definite age of the extract would correspond to the customary P_H-activity curve and show the variation in action with the hydrogen-ion concentration. The actions of the untreated extract after the different time periods are also given. The results are strictly comparable, being calculated to the same volume of undialyzed extract.

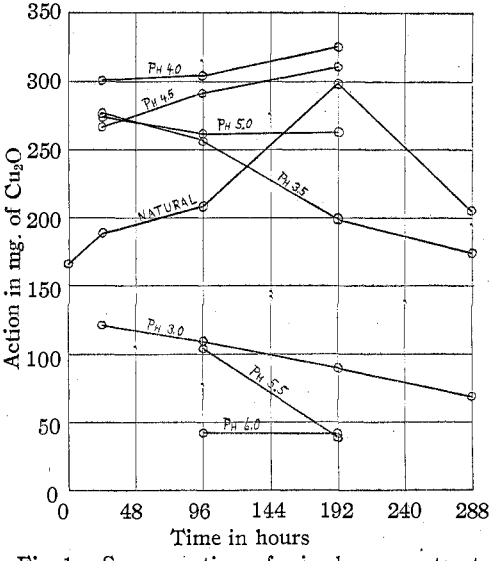

Fig. 1.—Sucrase actions of aging banana extracts tested at different hydrogen-ion concentrations (hydrochloric acid and sodium hydroxide).

The results in which hydrochloric acid and sodium hydroxide were added to fix the hydrogen-ion concentration showed a definite optimum for the actions at P_H 4.0 for the whole time period and an optimal zone between P_H 3.5 and 5.0 with very rapid falling off in more acid and more alkaline solutions. These confirm the results previously reported. The activities of the extracts on aging showed a small increase when tested at P_H 4.0 and 4.5, a small decrease when tested at P_H 5.0, and a greater decrease when

[4] W. M. Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, **1922**, Chapters V and VI.

tested at P_H 3.0, 3.5 and 5.5. The behavior of the extract on aging when tested without the addition of any substance to change or fix the hydrogen-ion concentration was striking. Its action was 80% greater after 196 hours than when tested immediately after preparation, and then fell off rapidly again. If a change in hydrogen-ion concentration is considered to be responsible for this increase in activity, then if the activities as found by the addition of hydrochloric acid and sodium hydroxide are taken as the standards, the P_H of the untreated juice as freshly prepared would be 3.2, or decidedly more alkaline than 5.0 (possibly 5.4), and in its most active state (at 196 hours) either 3.8 or 4.7. The possibility of the existence of the extract in the more acid condition may be considered to be eliminated. The change from P_H 5.4 to 4.7 would be more difficult to follow. At the same time, the indicator colors would be expected to show such a change. Looked at in a somewhat different way, if the highest activity found for the untreated juice were carried back to zero time, the addition of acid to bring the mixture very nearly to P_H 4.0, would be required to produce the same activity immediately, as is obtained after 196 hours at P_H 4.7. Further, considering the decrease following the maximum value would necessitate a Sörensen value between 3.0 and 3.5 if acid production were solely responsible for the change. In other words, the change in hydrogen-ion concentration is not the only factor involved in this increase in activity, if indeed it is involved at all. It may be noted also that if the P_H-action curves are plotted for the various times, the optimal zone is narrower after the longer time intervals. At 30 hours, the action at P_H 5.0 is slightly greater than at P_H 4.5. These points are most difficult to fix experimentally, and may be slightly in error.

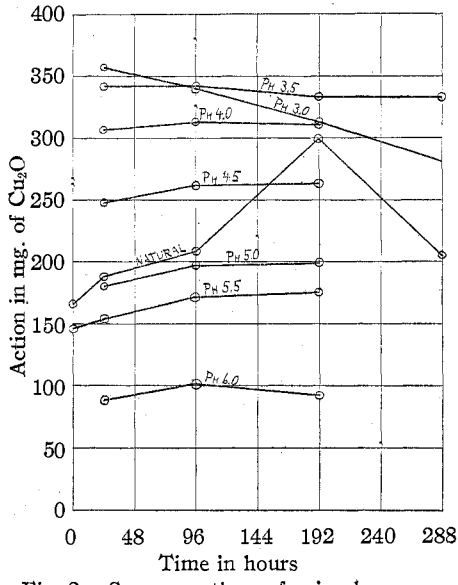

Fig. 2.—Sucrase actions of aging banana extracts tested at different hydrogen-ion concentrations (citrate buffer mixtures).

The extracts tested in the presence of citrate-buffer mixtures show a different picture. Tested after 24 hours, no optimum was found, the most acid mixture (P_H 3.0) showing the greatest action. On aging, the extract tested at P_H 3.0 showed marked decrease in action, that tested at P_H 3.5 little change, those tested between P_H 4.0 and 5.5 small increases, and that

tested at P_H 6.0 a slight increase followed by a slight decrease. At 96 hours and after longer times of aging, an optimum for the actions was shown at P_H 3.5. The untreated extract, on the basis of these findings, should have a P_H of 5.1 as prepared, and in its most active state a P_H of 4.2, if the change of activity is dependent on change in hydrogen-ion concentration as evidenced by the mixtures to which citrate buffers had been added.

It may also be pointed out that a comparison of the actions of the extract at different hydrogen-ion concentrations on the one hand with hydrochloric acid or sodium hydroxide added, on the other hand with citrate buffer mixtures added, shows at P_H 4.0 very nearly the same activity for both, at P_H 3.0 and 3.5 greater activities with citrate, at P_H 4.5 and 5.0 smaller activities with citrate, and at P_H 6.0 greater activity again with citrate.

The results just given form a fairly complete series and bring out certain relations clearly. They are characteristic of a number of similar experi-

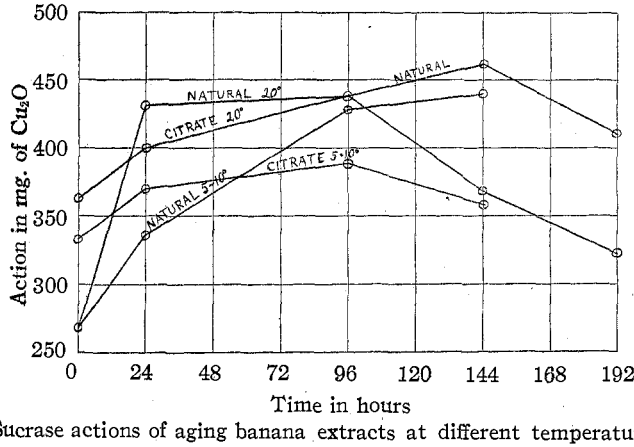

Fig. 3.—Sucrase actions of aging banana extracts at different temperatures tested in the presence of citrate (P_H 4.5) and with nothing added.

ments. Differences were observed with the different banana extracts with reference to the rate and amount of increase of action, etc., but the same general relations were noticed.

In Fig. 3 are shown the results found in the increase in activity of an extract aging at two different temperatures compared with the activities with citrate buffer, P_H 4.5, added before testing, using 1.5 cc. to 20 cc. of sucrose-extract mixture.

These results show clearly that the increase in activity was more rapid in the solutions kept at higher temperatures, in agreement with the results of Table II. As compared with the actions tested in the presence of the citrate buffer, the relations in the two cases were essentially the same as those shown in Fig. 2.

The fact that citrate buffer mixtures exerted such marked actions on the sucrase, made it desirable to test the behavior of a banana extract on aging, tested with different amounts of citrate added. The results are given in Table IV.

TABLE IV

SUCRASE ACTIONS ON AGING OF BANANA EXTRACT TESTED IN THE PRESENCE OF DIFFERENT AMOUNTS OF BUFFER MIXTURES AT SEVERAL HYDROGEN-ION CONCENTRATIONS

Buffer added P_H	Buffer mixture added to 20.0 cc. of enzyme-sucrose solution Nature	Cc.	Sucrase actions in mg. of Cu_2O per cc. of undialyzed extract incubated for 2.5 hours at 37.5° after standing at 5–10° for the indicated lengths of time 0 hours	120 hours	192 hours
3.5	Citrate	0.75	...	...	377
		1.5	...	...	377
		3.0	...	...	382
4.1	Citrate	0.75	...	391	342
		1.5	...	397	361
		3.0	...	400	366
4.4	Citrate	0.75	...	366	326
		1.5	350	377	336
		3.0	...	354	316
4.6	Citrate	0.75	...	326	...
		1.5	...	320	...
		3.0	...	294	...
4.6	Phosphate	1.5	...	239	...
Natural		...	175	334	330
5.1	Citrate	0.75	...	259	268
		1.5	208	244	233
		3.0	...	181	175
5.1	Phosphate	1.5	...	169	...
5.45	Citrate	0.75	...	256	242
		1.5	181	(124)	205
		3.0	...	...	146

These results show that in the more alkaline solutions the actions decrease markedly with increase in citrate concentration. Considering the concentrations of the citrate added, it does not seem probable that this decrease is solely a hydrogen-ion concentration change. These decreased actions must, therefore, be caused by the salts in the buffer mixtures added. At P_H 4.6 a similar action, but much less, is observable, but in the more acid solutions there appears to be no difference due to amount of added citrate. The variations here are irregular and may well be due to experimental errors.

The increase in action of untreated extract was 90% in this experiment. If this change were due to change in hydrogen-ion concentration only, it would mean, on the basis of the citrate experiments, a change from P_H 5.5 (or more alkaline) to about P_H 4.5. Such a change would surely be detectable by indicator measurements.

Two experiments with phosphate buffer mixtures[4] at P_H 4.6 and 5.1, in

each case 1.5 cc. being added to 15 cc. of sucrose solution and 5 cc. of enzyme extract in the customary way, which are shown in the table, indicated greater retarding actions than with the citrate mixtures. Some experiments with phthalate and acetate buffer mixtures also showed greater inhibitions.

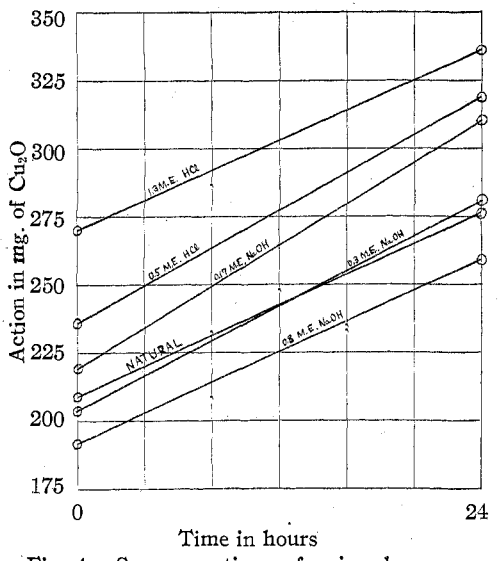

Fig. 4.—Sucrase actions of aging banana extracts tested after the addition of small amounts of hydrochloric acid and of sodium hydroxide.

It was observed that, under certain conditions of aging extract, addition of very small amounts of hydrochloric acid or of sodium hydroxide, which gave no indication of color changes with indicators, resulted in definite increases in sucrase action. The results of two experiments shown in Figs. 4 and 5 illustrate this.

It is obvious that change in hydrogen-ion concentration cannot account for the increased activities here, especially when alkali is added. These increases are not large, it is true, but they are marked. They were not found with every extract tested but were observed with a sufficient number, of which two are given, to make certain of the increase. The experiment given in Figs. 1 and 2 shows similar increases. It seems as if there is a certain state of ripeness of the banana, or age of extract, when this increase in activity of an extract may be observed. In Fig. 5 a definite increase is shown if the mixtures with the added acid or alkali were allowed to stand for 3 hours and then tested, but not if tested after 15 minutes.

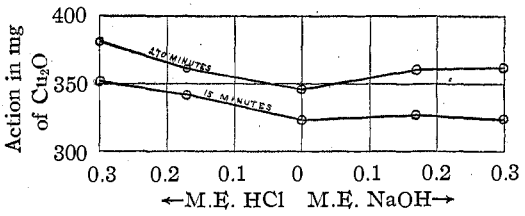

Fig. 5.—Sucrase actions of banana extracts tested 15 minutes and 270 minutes after the addition of small amounts of hydrochloric acid and of sodium hydroxide.

A number of additional factors were studied in connection with the increase in sucrase activity of the banana preparations, but with negative results. Thus, there was no change in titrable acid, with different indicators, on the aging and accompanying change in sucrase activity of the

preparations. Considerable protein material was present in the extracts, but no hydrolysis of peptide or similar linkages could be found by means of formol titrations. The amounts of reducing substances were not increased. The simpler sugars and other substances were not involved since the changes were observed with dialyzed extracts. The addition of small amounts of sodium chloride did not produce increased actions.

Discussion of Results

In the work described in this paper, no attempt was made to study the kinetics of the sucrase-sucrose reaction or to formulate mathematically the mechanism of the changes. Further, the times required for equal actions in the sucrose-hydrolysis reaction were not studied. For the purposes in view and the problems which were under investigation such careful comparisons were not essential. It was sufficient to determine the amounts of action occurring in definite time intervals.

The striking feature of the results is the spontaneous increase in sucrase action of the banana extracts on standing or "aging." This increase varied considerably in magnitude; in some cases the original activity was almost doubled. The state of ripeness of the banana at the time of extraction evidently influenced this subsequent increase. The increase was more rapid at higher temperatures than at lower. After reaching a maximum value in a certain number of days, the sucrase activity decreased again.

This increase in sucrase activity was independent of the composition of the solution used for extraction, and was not due to bacterial growth or cell decomposition accompanied by enzyme liberation.

The possibility that change in hydrogen-ion concentration occurring during the aging produces more favorable conditions for, and accompanies increase in, sucrase action, is the most obvious chemical explanation of the increase and must be considered most carefully.

Potentiometric methods could not be used for determining the hydrogen-ion concentrations of the banana extracts used in the experiments.[5] Indicators were therefore used throughout. As far as could be told, the banana extracts as prepared showed Sörensen values between 4.5 and 5.0. No satisfactory color differences could be obtained between these limits, and while the colors at P_H 4.5 and 5.0 were sufficient to fix these points, the possibility remained of acid production on aging which might change the P_H sufficiently to give increased sucrase action.

In order to fix the hydrogen-ion concentrations of enzyme preparations, buffer mixtures of definite hydrogen-ion concentrations and various compositions may be added. This method has been frequently used in determining the P_H-action curve for yeast sucrase and has apparently given satis-

[5] Similar difficulties were found with yeast sucrase preparations; Sörensen, *Compt. rend. trav. lab. Carlsberg*, **8**, 131 (1909). J. M. Nelson and W. C. Vosburgh, THIS JOURNAL, **39**, 810 (1917).

factory results. At the same time, the possibility must be kept in view, that the presence of added salts or other substances may influence the enzyme action of a preparation. While with preparations of the yeast sucrase such interfering actions appear to be small if not negligible, especially in the concentrations which are frequently used, they cannot be ignored completely, as brought out clearly in the recent work of Vosburgh.[6] The influence of added substances appears to be more marked with the banana sucrase as used in the present investigation than with the yeast sucrase. The P_H-action curves of the banana sucrase were found to be influenced by the age of the preparation, and the optimum actions to vary within certain limits, with this factor and the substances added to fix the hydrogen-ion concentration.

Although these variables exist and must be considered in the interpretation of the results, it was possible to show that if the increase in the sucrase action on the aging of the banana juice was due to a change in hydrogen-ion concentration to a more favorable reaction, a change of a magnitude very unlikely to escape detection would be required. Furthermore, it was also found possible to obtain increases in actions of extracts under certain conditions of aging by the addition of very small quantities of sodium hydroxide. This observation appears to rule out definitely the formation of acid on aging as the only factor in the production of more favorable conditions of hydrogen-ion concentration as an explanation of the increase in sucrase action. That the addition of electrolyte as such was not involved is evident because the juice was not entirely free from salts, and also because experiments in which small amounts of sodium chloride were added did not show increased actions.

Some possible explanations for the relations found may be suggested briefly. The increase in sucrase action may be explained as due to the fact that the conditions (physical or chemical) for the formation of the addition compound of enzyme and substrate or for its decomposition are favored so that the velocity of one or both of these reactions is increased. No evidence is available to test this view.

Another explanation would involve the formation of new enzyme from material present in the extract. In the ripening banana, the sucrase content has been found to increase,[1,7] Changes in enzyme contents of living matter are of common occurrence. It is, therefore, readily conceivable that the banana extract contains material which, in the absence of the life process, "spontaneously" forms new enzyme.

This is the simplest chemical explanation of the observations. The increase followed by a decrease may be readily accounted for by the fact that the banana sucrase in the extract loses its activity steadily, that the

[6] Vosburgh, THIS JOURNAL, **43**, 1693 (1921).

[7] E. M. Bailey, *ibid.*, **34**, 1706 (1912).

formation of new enzyme takes place rapidly at first and gradually decreases in rate as available material is used up, and that finally, destruction of enzyme is more rapid than formation of new enzyme.

The question may be raised, whether the increase in sucrase action (or possibly formation of new sucrase) is peculiar to banana extracts. Yeast sucrase has been studied more intensively probably than any other enzyme. Much has been done in connection with the increase in sucrase content in the living or growing yeast under suitable conditions of nourishment, but nothing appears to have been recorded as to increases in the yeast extracts apart from the life process. At the same time, the yeast sucrase has been studied, as a rule, only after considerable manipulation of the material. The properties of such preparations may have reached a certain state of constancy, especially since the yeast sucrase appears to be very much more stable than banana sucrase. Even marked differences in stability and action of such yeast sucrase preparations have been observed on careful study.[8] It is, therefore, possible that the properties of the yeast sucrase preparations as investigated in the majority of cases, differ in certain respects from the sucrase properties of the living yeast and of the materials freshly prepared from the yeast.

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Summary

The sucrase actions of banana extracts increased on standing from 40 to 100%, and then decreased.

This increase was independent of the composition of extracting solution or of the preservative present. Banana cells and bacteria were absent during the increase.

The increase in activity was not accounted for by change in hydrogen-ion concentration.

Experimental results of the sucrase actions at different hydrogen-ion concentrations and different ages of extract are given. Rise in temperature increased the rate of increase.

Possible explanations of the increase in activity are discussed.

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[8] Nelson and Hitchcock, THIS JOURNAL, **43**, 2632 (1921).