STUDIES ON ENZYME ACTION.

XIX. THE SUCROLYTIC ACTIONS OF BANANAS.

BY K. GEORGE FALK AND GRACE McGUIRE.

(From the Harriman Research Laboratory, The Roosevelt Hospital, New York.)

(Received for publication, February 3, 1921.)

INTRODUCTION.

The change of starch into simpler carbohydrates such as glucose, sucrose, etc., or the reverse reaction, is a general phenomenon which accompanies the growth and development (so called ripening) of many edible fruits and also of a number of vegetables. For example, the starch of apples and of bananas in the maturing of the fruit becomes converted into simpler carbohydrates, while the sugars of maize kernels and of peas are converted into starches. In the belief that enzymes and enzyme actions play an important, if not a predominating, role in these chemical changes, a study was begun of the enzymes which might be expected to be involved in these reactions. The banana (Musa sapientum) was chosen as a typical example of a fruit in which such changes occur because of the possibility of readily obtaining large quantities of the unripe fruit, the rapidity with which it can be made to ripen, the fact that it ripens when separated from the growing plant, and the extensive change from starch into glucose, invert sugar, and sucrose which accompanies the ripening.

Previous Work.

For the purpose in view, the chemical composition of the unripe and ripe bananas is of interest. The results given by Gore are perhaps the most satisfactory and may be quoted as follows: As a result of ripening a number of bananas, a loss in weight of 3.88 per cent was

1 Cf. Sherman, H. C., Chemistry of food and nutrition, New York, 1918, 12.
observed. When unripe, the fruit consisted of 41.72 per cent peel and 58.28 per cent pulp; when ripe, 37.85 per cent peel and 62.15 per cent pulp. During ripening, the main changes in the peel consisted of a decrease of 5 per cent in water content and the transformation of two-thirds of the starch into sugar. In the pulp, the starch content changed from 13.15 to 2.40 per cent, the reducing sugars from 0.37 to 10.34 per cent, and the sucrose from 0.38 to 1.52 per cent, while the water content increased 1.6 per cent.

Enzyme studies on bananas have been carried out from time to time. The most complete investigations were published by Tallarico, who reported the presence of sucrase, amylase, protease, and catalase, the absence of lipase, while the results for tyrosinase were not conclusive, and by Bailey, who found amylase, sucrase, raffinase, protease, lipase, and peroxidase, but not maltase, dextrinase, or lactase. Sucrase and amylase are mainly of interest in connection with the present work. Both Tallarico and Bailey proved qualitatively the presence of sucrase in bananas, considerably more in ripe than in unripe ones. With regard to the amylase, the results were not so satisfactory. The errors in the method used by Tallarico apparently were as great as the observed actions. Bailey's results were obtained with the iodine test under certain conditions.

Methods of Testing.

The amount of amylase or of sucrase action was determined in most of the experiments by the estimation of the reducing sugars formed. In carrying out the estimations, from 2 to 10 cc. portions of the mixtures tested were used, depending upon the amount of reducing substance present.

In a few experiments the starch-splitting actions were followed by adding iodine dissolved in potassium iodide solution to the mixtures. Sodium hydroxide and hydrochloric acid solutions were employed to bring the mixtures to definite hydrogen ion concentrations. In

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3 Tallarico, G., Arch. farm. sper. e sc. aff., 1908, vii, 27, 49.
some of the series of sucrase actions, buffer mixtures were added directly to the enzyme-substrate mixtures. The indicators recommended by Clark and Lubs were used.\(^7\)

Since a number of different procedures were employed to obtain the enzyme preparations from the bananas, these will be given in connection with the results obtained.

**General Properties of Unripe and Ripe Bananas.**

Some of the general properties of the unripe and ripe bananas\(^8\) will first be given. The pulp of the unripe banana adheres to the peel, making peeling difficult, while with the ripe banana the peel is easily removed. The pulp of the green banana is more fibrous in character and mashes with difficulty, in marked contrast to that of the ripe banana which readily forms a wet, soft paste. On mixing with water, the pulp of the former does not form a homogeneous mass, that of the latter does. In extracting (grinding) repeatedly with small portions of water and squeezing through muslin, a white cloudy liquid and a large quantity of insoluble residue are obtained from unripe banana pulp, while from ripe banana pulp most of the mixture can be squeezed through the muslin leaving only a small amount of very soft residue. The pulp of the unripe banana differs from that of the ripe banana also, in containing a sticky substance which discolors the hands.

The qualitative factors which have been given, as well as the color of the peel, serve to show the state of ripeness of the banana. More quantitative relations have been obtained by use of the "coefficient of ripeness" which represents the ratio of weight of pulp to weight of peel at the different stages.\(^9,10\) This ratio increases in value as ripening proceeds.\(^1\) The decrease in the percentage of starch present either in the peel or in the pulp might also be used as a quantitative measure of the state of ripeness.


\(^8\) We wish to thank the Fruit Dispatch Company for supplying the greater part of the bananas used in this investigation.
Enzyme Tests with Unripe Bananas.

The enzyme tests with unripe bananas can be summarized briefly; since (a) the general relations found were the same as with ripe bananas, except that (b) greater enzyme actions were obtained with ripe banana preparations than with unripe, and (c) the nature and properties of the unripe banana rendered working with it more difficult and less satisfactory.

The methods of studying the unripe banana pulp were as follows:

1. Pulp extracted with equal weight of water, filtered through muslin. Filtrate and residue (to which an equal quantity of water had been added) tested at different hydrogen ion concentrations in 1 per cent sucrose (also more concentrated) and starch solutions for 18 hours at 35°C.
2. Same as Method 1 with one-half the weight of water.
3. Same as Method 1 with one-quarter the weight of water kept between 5° and 10°C. during the preparation.
4. Pulp ground in food chopper, no water added.
5. Pulp and peel ground together in food chopper, no water added, and paste tested (mainly for amylase).
6. Pulp ground with twice its weight of 95 per cent ethyl alcohol, filtered through heavy muslin, and centrifuged. Residue ground with half its weight of water, filtered through muslin, centrifuged, and residue and liquid tested.

It may be pointed out here that upon the addition of Lintner starch solution to unripe banana extract, a precipitate was immediately formed. With ripe banana extracts there was no precipitate. With the unripe extract filtered through paper, upon the addition of Fehling's solution to determine the enzyme actions, a gelatinous precipitate was formed. Only small portions could therefore be used for the tests as otherwise filtration through the Gooch crucibles was difficult. No such difficulty was experienced with ripe banana extracts. Toluene was used as preservative throughout these experiments.

The general results of the enzyme tests were as follows: For amylase action, in no case were the results such as to show definitely the presence of this enzyme. In a few experiments small apparent
actions were observed, but, in view of possible experimental errors, these apparent actions were not of sufficient magnitude to prove conclusively the presence of such an enzyme. The object of the preliminary treatment with alcohol (Method 6) was for the purpose of dissolving possible inhibiting substances, such as tannin, with the simultaneous precipitation of the enzyme in order to obtain the latter separated from inactivating soluble material. The tests for sucrase were positive, considerable action being obtained. Since the ripe banana could be handled more readily, a more extended and quantitative examination of the sucrase from this source will be reported.

A few of the results with the unripe banana preparations are given to show the nature of the actions. 20 gm. of pulp obtained by Method 4 with 10 cc. of 2 per cent starch solution after 21 hours at 32°C. gave reducing substances corresponding to 5.0 mg. of Cu₂O per gm. of pulp, and with 10 cc. of 20 per cent sucrose solution similarly, the reducing substances corresponding to 246 mg. of Cu₂O per gm. of pulp. 20 gm. of pulp plus peel mixture obtained by Method 5 treated similarly gave with the starch no reducing substances, and with the sucrose reducing substances corresponding to 203 mg. of Cu₂O per gm. of pulp plus peel.

The results of Bailey⁴ on the action of air on the green banana pulp were confirmed, no amylase being obtained by this treatment.

**Soluble Sucrase Preparation from Ripe Bananas.**

Soluble and insoluble sucrase preparations were obtained from ripe bananas. As a result of a number of different methods of extraction, the following procedure was found to give the most satisfactory soluble preparation. The banana pulp was ground rapidly in a food chopper, the finest cutter being used, then mashed in a porcelain mortar with a wooden potato masher with normal sodium chloride solution (100 cc. for each 400 gm. of pulp). Toluene was added and the mixture then filtered through paper. The filtrate was dialyzed 18 to 24 hours in collodion bags against running water to remove the salt, soluble dialyzable carbohydrates, and other products. The resulting solution was used in the sucrase experiments.
A number of experiments were carried out in which the treated pulp was centrifuged and the supernatant liquid dialyzed and used, or where the treated pulp was squeezed through muslin and then centrifuged. The properties of the sucrase solutions so obtained were essentially the same, but the above procedure was the one finally adopted for studying the soluble banana sucrase.

The following methods of extraction did not yield preparations so satisfactory as the salt treatment described: Extraction with one-fourth weight or equal weight of water (fairly active preparations, in one case 2½ times as much action was obtained by the salt extraction as by the water extraction); extraction of small portions of banana with ten times the weight of water or salt solution; autolysis, followed by filtration, etc., as in the preparation of yeast sucrase⁹ (slightly active filtrate obtained); and grinding in a ball mill for a long period of time (inactive filtrate).

It may be mentioned that repeated extractions did not offer any appreciable advantage over a single treatment. A small amount of active material could be obtained on a second extraction as compared with the first extract. Extracting for longer periods of time did not give appreciably more active solutions.

The following results were obtained in testing at different hydrogen ion concentrations. 10 cc. of the salt-extracted dialyzed solution plus 10 cc. of a 20 per cent sucrase solution were incubated for 2½ hours at 35°C. The actions are given in terms of mg. of Cu₂O produced by the action of 1.0 cc. of original undialyzed filtrate corrected for blanks.

<table>
<thead>
<tr>
<th>pH</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
<th>5.5</th>
<th>6.0</th>
<th>6.5</th>
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<tbody>
<tr>
<td>Actions</td>
<td>183</td>
<td>481</td>
<td>489</td>
<td>478</td>
<td>458</td>
<td>375</td>
<td>135</td>
<td>52</td>
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Fig. 1 shows these results graphically. There is a zone of maximum action between pH 3.5 and 4.5, with rapid drops beginning between 3.5 and 3.0 and at 5.0. This optimum zone corresponds to that observed with yeast sucrase solutions, where the zone is of different widths in various experiments and under different conditions,¹⁰ and with

potato sucrase. The exact optimum pH is difficult to determine in any case because of the flat portion of the curve, but with soluble banana sucrase it is not far removed from 4.0 at 35°C. for 2½ hours action. The method of presenting these results is not the most satisfactory but it shows definitely the optimum conditions. Not
enough data were obtained to calculate the velocity constants satisfactorily, assuming that such constants would be obtained. In order to determine the different times for the same action, further assumptions would have to be introduced. The hydrogen ion concentrations of the solutions did not change during the actions.

The amounts of sucrase action with the same quantity of enzyme material at different intervals of time were studied. The results are given in terms of mg. of Cu₂O obtained, calculated back to 1 cc. of original extract with 2.5 and 5 per cent sucrose solutions. The hydrogen ion concentration was that of the natural juice, not far removed from the optimum.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>2.5 per cent sucrose solution</th>
<th>5.0 per cent sucrose solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>134</td>
<td>150</td>
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<tr>
<td>3</td>
<td>220</td>
<td>226</td>
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<tr>
<td>4</td>
<td>224</td>
<td>319</td>
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<tr>
<td>5</td>
<td>228</td>
<td>394</td>
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<td>6</td>
<td>218</td>
<td>395</td>
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<td>24</td>
<td>230</td>
<td>393</td>
</tr>
</tbody>
</table>

These results are shown graphically in Fig. 2. The one point at 6 hours with 2.5 per cent sucrose solution is evidently incorrect. The experimental errors are magnified by the calculations, since the difference indicated by 228 and 218 was caused by a difference in the weighings of 2.3 mg. of Cu₂O.

These results bring out the following facts. The action is a linear function of the time, the amounts hydrolyzed being proportional to the time of action until practically all the sucrose was hydrolyzed. The action with 5 per cent sucrose solution was only slightly greater than with the 2.5 per cent solution, indicating that the sucrase was nearly saturated in the more dilute solution. However, the total action for the more concentrated solution was not twice as large as for the dilute solution in the limited times used. The products of the reaction evidently played a part here, interfering with the action of the sucrase. These results are similar to those obtained in the more extended investigations of yeast sucrase by others.

Active precipitates were obtained by the addition of alcohol or of acetone to the sucrase solution. These did not form clear solutions again, but showed considerable actions when tested as suspensions.

\(^{11}\) Similar relations within certain limits have been observed with yeast sucrase. Nelson, J. M., and Vosburgh, W. C., *J. Am. Chem. Soc.*, 1917, xxxix, 790. Michaelis and Rothstein.\(^{10}\)
Long continued treatment with alcohol appeared to inactivate the enzyme. These preparations were not studied further since the con-

Fig. 2. Time-action curves of soluble sucrase preparation; (a) 2.5 per cent and (b) 5.0 per cent sucrose solutions.

version of soluble into insoluble sucrase preparation in a different way appeared to be of more direct interest.
Insoluble Sucrase Preparation from Ripe Bananas.

It was found that the water (or salt solution) insoluble residue from the ripe banana pulp possessed considerable sucrose-hydrolyzing action. 12

2,760 gm. of pulp were ground with 700 cc. of N sodium chloride solution, 16 liters of water added, thoroughly stirred, allowed to stand over night at room temperature, and filtered through paper. The moist residue was treated with 10 liters of water similarly, and the residue (drained more thoroughly than the first residue) treated again with 6 liters of water and then with 8 liters. The filtrate from the last gave no sucrase action. The residue was dried by grinding twice with alcohol and then with ether. 0.75 gm. of this residue tested in 20 cc. of a 10 per cent sucrose solution gave reducing substances in 4 hours at 35°C. producing 355 mg. of Cu₂O per 0.10 gm. of residue.

The possibility that the cell walls of the banana pulp were not broken and that the sucrase was retained within the cells was tested a number of times by using different methods of grinding and extracting. Vigorous grinding in a mortar with sand as well as long continued grinding in a ball mill, also followed in some cases by grinding in a mortar, gave, after thorough extraction, active insoluble residues. In place of filtering through paper, the residue was also obtained by centrifuging or by filtering through asbestos, and showed similar actions. It was obtained as a grayish brown powder by grinding twice with 95 per cent alcohol, separating by centrifuging, and finally washing with ether and drying on filter paper at room temperature. This residue gave a nitrogen content of nearly 1 per cent.

A series of determinations with the centrifuged moist residue at different hydrogen ion concentrations gave an optimum action between pH 4.0 and 4.5 with a rapid falling off beyond 6.0. In view of the character of the material, a more careful determination was not made. The results showed an optimum similar to that of the soluble sucrase preparation which was the main question involved.

12 Euler and Svanberg (Euler, H., and Svanberg, O., Z. physiol. Chem., cvii, 269) recently studied the sucrase actions of the residue from autolyzed and extracted yeast.
Conversion of Soluble into Insoluble Sucrase Preparation.

The finding of soluble and insoluble sucrase preparations or materials showing sucrase actions led to further developments. The optimum action for both was found to be at approximately the same pH (nearly 4.0). This raises the question, which was developed at some length in another connection, as to whether a definite enzyme action was connected with a certain molecule or with a certain group which may be present in different molecules. If the latter view is adopted tentatively, the same active sucrase grouping would be present in the one case in a soluble molecular species, and in the other case in an insoluble molecular species. This point of view simplifies in some ways the consideration of the experiments to be described in this section.

That sucrase-carrying substances of different solubilities are present in banana pulp is shown by the fact that aqueous extraction yields one such, normal sodium chloride solution extraction following the aqueous extraction another, and the residue after repeated extraction a third. To judge from the hydrogen ion concentration for optimum action these sucrase actions are identical.

The most active and satisfactory soluble preparation was obtained by the salt extraction as described. The sodium chloride and the soluble simple sugars, etc., were removed by dialyzing over night in collodion bags against running tap water. The volume increased as a rule about 60 to 80 per cent. If the dialyzed sucrase solution was further dialyzed in a fresh collodion bag for 24 hours against tap water, the volume did not increase but a gel separated. The hydrogen ion concentration of the mixture did not change in the course of dialysis on the 2nd day. The gel could be filtered out readily by means of filter paper. The filtrate from the gel did not show sucrase action but the gel showed very marked activity. The gel was ground with alcohol twice and ether once and allowed to dry in the air at room temperature. It did not dissolve in water, but formed a gel with it. A suspension showed the following activity: 0.05 gm. of material in 20 cc. of a 10 per cent sucrose solution in 4 hours at 35°C. gave reducing substances producing 457 mg. of Cu₂O per 0.01 gm. of

Falk, K. G., The chemistry of enzyme actions, New York, 1921, 81.
solid preparation. It was therefore about fifteen times as active as the insoluble sucrase preparation obtained directly from the banana pulp. It showed a nitrogen content of 4.4 per cent; that is, about four times as large as that of the latter.

**Amylase Results with Ripe Bananas.**

The amylase tests carried out with Lintner starch or with banana starch, in which the amounts of reducing sugars were determined, did not give definite evidence of the presence of a starch-splitting enzyme. These tests were carried out with extracts and suspensions prepared as described in the sucrase experiments. Before determining the amounts of reducing sugars formed, the mixtures were filtered through paper and an aliquot of the filtrate taken. Undialyzed extracts gave considerable blanks which increased on incubation because of the sucrase action on the sucrose contained in the banana. Dialyzed extracts gave little or no blanks. Isolated results at times showed an apparent action, but the results were not consistent enough or large enough to warrant the statement of a definite saccharogenic action on the starch. The mixtures were tested at different hydrogen ion concentrations and also in the presence of salt. The solid residue from the extractions behaved similarly.

In view of Bailey's positive results with the iodine test (amyloclastic in place of saccharogenic actions) and since he found more marked actions with ripe than with unripe banana pulp, the reaction was studied in this way also. The method described by him was followed as closely as possible. It was found that, with the suspensions of banana pulp on incubation with Lintner starch, precipitates settled to the bottom of the tubes; that if these mixtures after incubation were tested with iodine after filtration, the filtrate gave no starch reaction but the precipitate on the paper became blue; that, if the whole mixture without filtering was shaken and tested with iodine, the precipitate was colored blue and settled leaving the supernatant liquid colorless. It is probable, though not altogether clear from the description of his experiments, that Bailey filtered or decanted these mixtures before testing with iodine. If that was the case, the amyloclastic actions which he described are open to ques-
Repeating his work, using the most satisfactory method of obtaining active preparations which he described, as well as extracts obtained as described with the sucrase experiments and incubating with 1 per cent Lintner starch solution for 24 to 40 hours at 30°C to 35°C., it was found that no amylolastic actions as marked as those described by Bailey were obtained. The greatest change which was observed in any of the tests was a change from the deep blue iodine starch reaction to a bluish violet color test. The amylolastic actions may therefore be said to be extremely small if present at all. In all these tests, toluene was used as preservative.

DISCUSSION.

The change of soluble sucrase material into insoluble during the simple treatment of dialysis raises the question of the state of the sucrase in the growing banana. On grinding to a pulp and extracting, soluble and insoluble preparations were obtained. This treatment may, however, have been sufficient to change materially the properties of the substances originally present. It is therefore conceivable that the sucrase may be present as a completely soluble substance in the ripening fruit and that the differences in solubility observed were due entirely to the treatment to which the fruit was subjected. It must also be recalled that toluene was added whenever the treatment extended over a greater period of time than a few minutes and that this toluene may exert a definite influence, possibly of a coagulating nature, upon the substances present.

This change in solubility was also observed when the bananas were ground in a ball mill with toluene present. 8 hours grinding followed by extraction gave a certain amount of soluble sucrase although less than was obtained by grinding in a mortar for a shorter period of time. Grinding for a week, however, resulted in the extract showing no sucrase action at all. The residue showed marked activity. It is probable that here, too, the soluble sucrase was converted into insoluble material. The reasons or causes for this change, whether due to dialysis and removal of certain products, the action of toluene, or other cause, are not known but will be investigated further.

14 Cf. similar observations with potato amylase.
The change of soluble into insoluble sucrase upon the simple treatments described illustrates the sensitive character of materials occurring in living matter. In this case, the enzyme property is not destroyed but a different property (solubility), which can be traced by following the enzyme action, is changed.

Sucrase has been generally taken to be one of the most hardy of the enzymes. It is not inactivated as rapidly as most other enzymes or under conditions which cause these to lose their activity completely. On the other hand, amylase has been found to be highly sensitive to outside influences and to be destroyed under comparatively simple conditions. It is possible that the simple treatments, which changed the properties of the material carrying the sucrase action without destroying that action, may destroy the amylase action which would be expected to be present in the ripening banana. Such an explanation would account for the failure to obtain a definite and marked amylase action in banana pulp preparations or extracts.

The question of the conversion of starch into simpler carbohydrates may be considered further. For such an action in living, growing matter, the presence of the enzyme amylase, on the basis of past experience, is required. Experimental tests have not shown conclusively that this enzyme is present in the banana. Reasoning on the basis of the active amylase preparations described by Sherman it is possible that a minute quantity of such material would suffice to produce the changes observed in the banana, but, on the other hand, it should be possible to obtain experimental evidence of the presence of such an enzyme. It is also possible that substances are present in the banana which when brought into close contact, as by grinding, with the enzyme material, inhibit the action. It is possible to imagine a cellular structure of such nature that enzyme material and inactivating substance (possibly tannin) are separated in the fruit in the form and shape in which it occurs in nature and that artificial treatment involving destruction of the cell structure is accompanied or followed by profound changes in the cellular contents.

There is, however, another possible view-point. In ripening "The most conspicuous change is the long-recognized conversion of

starch into sugars. It is most rapid while the fruits are turning from green to yellow. During this period the respiration rate increases manyfold, becoming greatest at the time when the rate of starch hydrolysis is most rapid. Bailey showed that the banana ripened in the presence of oxygen, but not in gases such as hydrogen, carbon dioxide, etc. It is therefore possible that the ripening process, or the breaking down of the starch, is not merely an amylolytic action as this is commonly understood, but involves a simultaneous or preliminary oxidation reaction.

SUMMARY.

A number of different methods of treatment of unripe and ripe bananas for the purpose of obtaining and studying sucrolytic and amylolytic enzymes are described.

No conclusive evidence of the presence of an amylase could be obtained in any of the preparations.

The sucrase of unripe and ripe bananas was studied more extensively. With ripe bananas, both soluble and insoluble sucrase preparations were obtained. Conditions for converting the soluble into an insoluble form were found. The actions of the sucrase preparations as far as the hydrogen ion concentration for maximum action and the time-action relation are concerned are similar to the behavior of the yeast and the potato sucrase.