[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Co-Ferments and Banana Respiration

BY J. M. NELSON AND CATHARINE HEMPERLY

The metabolism of respiration in the case of the higher plants plays a dominating role in the ripening and storage of fruit, the curing of hay, tobacco, grains, etc. Although considerable work has been and is being done on the chemistry of respiration as it is related to fermentation by yeast and to the glycolysis in muscle tissue, our knowledge along these lines concerning plant respiration is still relatively meager. The present paper is an account of the study of glycolytic activators present in the pulp and juice of bananas.

The presence of zymasic activators in plant material, such as pea meal, has been shown by Bodnar and Hoffner.[1] Euler and Steffenburg[2] added boiled apple juice as well as a boiled aqueous extract of the fruit to some yeast apo-zymase preparation, but observed practically no stimulation of zymasic activity. From other results, however, they concluded that these experiments did not necessarily show the absence of cozymasic activators, but rather the presence of inhibitors, probably phenolic in character, which tend to mask the presence of the activators in the extracts from apples.

The present authors, in connection with some work on the chemical changes involved in the ripening of bananas, find the juice from this fruit to contain co-ferments. The presence of these co-ferments or activators in bananas was established by much the same procedure as that used in the case of yeast and muscle tissues. Boiled aqueous extracts of the fruit were added to a mixture of yeast apo-zymase, glucose, sodium phosphate, hexose diphosphate and water, contained in the reaction flask of a Warburg–Barcroft respiration apparatus and the rate and amount of carbon dioxide given off measured.

Apo-zymase Preparations.—These were obtained by stirring several grams of air or acetone dried brewer's yeast with twenty times its weight of water for fifteen minutes, centrifuging, and repeating the washing. For some lots of yeasts, four washings were necessary in order to remove most of the cozymase. Preparations obtained in this way produced very little fermentation when mixed with solutions containing glucose, sodium phosphate and hexose diphosphate. Dried pea meal was also used without washing in some instances as the apo-zymase preparation since very little fermentation occurs when the meal is added to a solution of glucose, disodium phosphate and hexose diphosphate The yeast apo-zymase preparations were more active than the pea meal and therefore mostly used in this work.

Method for Determining Accelerating Effect of the Co-ferment.—The Warburg–Barcroft apparatus[3] was used; described briefly, it consisted of a 50-cc. flask, containing the reaction mixture of co-ferment, ferment and substrate, attached to a manome-

(1) Bodnar and Hoffner, *Biochem. Z.*, **165**, 145 (1925).
(2) Euler and Steffenburg, *Z. physiol. Chem.*, **175**, 38 (1928).
(3) Warburg, *Biochem. Z.*, **142**, 317 (1923); **152**, 51 (1924); **214**, 5 (1929).

ter for measuring the carbon dioxide evolved. The reaction flask was immersed in a constant temperature bath, kept at 35° and shaken continuously by a mechanical device attached to a motor.

Co-ferment Preparations from Bananas.—The most convenient and effective preparations of co-ferment were made by grinding in a mortar, fresh yellow ripe bananas with half their weight of water, centrifuging and boiling the supernatant liquid for two to three minutes. Green bananas can also be used for preparing the co-ferment extract, or banana juice obtained by mixing banana pulp with sand and infusorial earth and pressing the mixture in a hydraulic press using about 2000 pounds pressure. Some extracts were also prepared using commercial banana flour.

Activity of Co-ferment Preparations.—Apo-zymase preparation made from 4 g. of dried brewer's yeast. To this was added 10 cc. of 30% glucose solution, 4 cc. of 0.66 *M* sodium phosphate solution (P_H 6.9), and 3 cc. of a 4% hexose diphosphate solution. The mixture (P_H 6.4) was then diluted to 40 cc. and 2 cc. of the resulting suspension was pipetted into respiration flasks containing the following material: (1) one cc. of distilled water; (2) one cc. of ripe banana extract (prepared as described above); (3) one cc. of green banana extract; (4) one cc. of extract from zymin (acetone dried yeast extracted as described above); (5) one cc. of extract from ripe bananas (20 g. of ripe banana pulp ground in a mortar with 100 cc. of acetone, and repeated twice more when a fine powder was obtained. This powder was extracted with ten times its weight of water); (6) half a cc. of extract as in 2 and 0.5 cc. of extract as in 3.

Time in minutes	Cc. of carbon dioxide given off					
	1	2	3	4	5	6
30	...	0.34	0.06	1.78	0.28	0.21
60	...	.57	.14	3.41	.58	.38
90	Trace	.71	.19	4.29	.81	.47

The values for the ripe banana extracts, columns 2 and 5, are larger than those for the extract from green bananas, column 3, indicating the presence of more co-ferment in the ripe than in the green fruit. The values in column 6 correspond to about the mean for the values in columns 2 and 3, indicating that there was no inhibitor present in the green banana extract causing the low values in column 3.

The accelerating influence of the co-ferment from banana on yeast apo-zymase action is considerably less than that of the yeast cozymase as can be seen by comparing columns 2 and 5.

Stability of the Banana Co-ferment.—A water extract of ripe banana was prepared by grinding the pulp with an equal weight of water, centrifuging and boiling the supernatant liquid for two to three minutes. This extract had P_H 5.0. The extract was subjected to the following conditions. (A) To a portion of the extract sufficient hydrochloric acid was added to make the solution about 0.1 *M* in HCl and P_H about 1. This acid solution was allowed to stand at room temperature for an hour and then neutralized with a solution of sodium hydroxide until the P_H was 5.2. (B) A sodium hydroxide solution was added to another portion of the extract until the P_H of the solution was 9.4 This was allowed to stand at room temperature for an hour and then neutralized with hydrochloric acid until the P_H became 4.8. (C) A third portion of the above banana extract was evaporated to a sirupy mass on the water-bath (one hour duration of heating) and then diluted with water to the original volume. (D) A fourth portion of the banana extract was brought to P_H 6.4 with dilute sodium hydroxide, then heated on the water-bath as in C for one hour, and finally diluted to the original volume. Five cc. portions of a mixture, consisting of yeast apo-zymase, glucose, sodium phosphate and hexose diphosphate, similar to apo-zymase mixture in previous experiments, were pipetted into respiration flasks containing the following: (1) two cc. of water;

(2) two cc. of solution A; (3) two cc. of solution B; (4) two cc. of solution C; (5) two cc. of solution D, and (6) two cc. of original extract.

Time in minutes	Cc. of carbon dioxide evolved					
	1	2	3	4	5	6
60	0.00	0.51	0.09	0.28	0.18	0.64
120	.00	.85	.14	.55	.34	1.12

The above figures show that the co-ferment in the banana extract is greatly inactivated by alkali (solution B and column 3) and by heating at P_H 6.4 (solution D and column 5). On the other hand the co-ferment is more resistant toward acid and in both of these behaviors the banana co-ferment resembles yeast cozymase.[4]

Relation between Amount of Co-ferment and Amount of Carbon Dioxide Evolved.—Some ripe banana extract, prepared as in the previous set of experiments, was brought to P_H 6.2 and then 1 cc. of 0.66 *M* sodium phosphate added, giving a solution of P_H 6.4. Five cc. of a yeast apo-zymase mixture, similar to that used in the previous set of experiments, was pipetted into each of a series of respiration flasks which contained, respectively: (1) six cc. of water (the water used in this set of experiments contained the same amount of added sodium phosphate as the banana extracts); (2) one cc. of banana extract and 5 cc. of water; (3) two cc. of extract and 4 cc. of water; (4) four cc. of extract and 2 cc. of water; (5) five cc. of extract and 1 cc. of water; (6) six cc. of extract.

Time in minutes	Cc. carbon dioxide evolved					
	1	2	3	4	5	6
30	0.03	0.03	0.06	0.25	0.38	0.51
60	.05	.07	.13	.43	.64	.84
120	.08	.13	.22	.63	.91	1.20

These values show that there is a gradual increase in the volume of carbon dioxide given off as the amount of added extract is increased.

Influence of Magnesium Chloride and Acetic Aldehyde.—Five cc. of yeast apo-xymase mixture, prepared as in previous experiments, was added to respiration flasks containing the following material: (1) two and 0.6 cc. of water; (2) two cc. of ripe banana extract (prepared as in previous set of experiments) and 0.6 cc. of a 2% aqueous solution of acetic aldehyde; (3) two cc. of ripe banana extract and 0.6 cc. of water; (4) two cc. of extract and 0.6 cc. of 2% magnesium chloride solution.

Time in minutes	Cc. of carbon dioxide evolved			
	1	2	3	4
55	..	0.281	0.217	0.352
105	..	.576	.523	0.814
145	0.00	.857	.814	1.221

The magnesium chloride has an accelerating effect on the reaction, while the acetic aldehyde has practically none.

The Co-ferment in Banana Extract Diffuses through a Collodion Membrane.—Some ripe banana extract and a few drops of toluene were placed in a collodion dialyzing bag and the latter immersed in a large beaker of water. After dialyzing for two days, the liquid outside of the bag was concentrated in an evaporating dish on the steam-bath to the original volume of the extract. The activities of the concentrated liquid, the contents of the dialyzing bag, and the original banana extract were determined in the usual way. Two cc. of yeast apo-zymase mixture (prepared as in the previous experiments) was added to each of a series of respiration flasks containing the following material: (1) two cc. of water; (2) two cc. of the contents of the dialyzing bag after

(4) Euler and Myrbäck, *Z. physiol. Chem.*, **133**, 260 (1924).

dialysis; (3) two cc. of concentrated liquid from outside the bag; (4) two cc. of the original banana extract.

Time in minutes	Cc. of carbon dioxide evolved			
	1	2	3	4
30	0.03	0.04	0.28	0.29
60	.06	.06	.53	.55
90	.06	.06	.63	.68

The values in columns 3 and 4 show that practically all of the co-ferment diffused through the collodion membrane.

Other Enzymes Belonging to the Glycolysis Chain. Phosphatase.—To test for the presence of phosphatase, a few drops of toluene, 2 cc. of a 4% sodium hexose diphosphate solution, and 5 cc. of water were added to 23 g. of ripe banana pulp, and the mixture ground in a mortar. Twenty cc. of a 6% trichloroacetic acid solution was added to the mixture and stirred, to destroy enzymes and precipitate proteins, and then made up to 250 cc. volume with water. After the mixture thus prepared had stood overnight, it was filtered and the inorganic phosphorus determined by Martland and Robison's modification of Briggs' method.[5] Two cc. of the filtrate contained 0.057 mg. of inorganic phosphorus.

Another lot of pulp (24 g.), hexose diphosphate, toluene and water, similar to that above, was prepared and allowed to stand overnight (eighteen hours) before adding the trichloroacetic acid, etc., so as to permit any phosphatase present to act on organic phosphates. Two cc. of this filtrate contained a much larger amount of inorganic phosphorus, 0.208 mg., thereby showing the presence of phosphatase.

In a similar way the banana extract was also found to contain phosphatase, showing this enzyme to be present in a soluble form.

Phosphatese.—To see whether any phosphoric acid esterifying enzyme was present in the banana, 20 g. of pulp was ground with a few drops of toluene, 2 cc. of 0.66 *M* sodium phosphate and 5 cc. of water (P_H of resulting mixture about 5.8). To determine the amount of inorganic phosphorus present, 20 cc. of 6% trichloroacetic acid were added, the mixture made up to 500 cc. with water and filtered. One cc. of this filtrate contained 0.094 mg. of inorganic phosphorus. A similar preparation which was allowed to stand eighteen hours before adding the trichloroacetic acid gave practically the same amount of inorganic phosphorus per cc., 0.092 mg. The difference between these two values is practically within the limits of experimental error and hence cannot be taken as evidence for the presence of any phosphorylating enzyme in the banana. The authors, however, do not wish to state that any enzyme of this kind is absent, but rather that possibly the method used in testing for its presence may not have been suitable.

Carboxylase.—To test for carboxylase banana pulp was ground with 0.66 *M* sodium phosphate solution, P_H of mixture about 6.4, and equal amounts of the mixture were placed in each of two respiration flasks. To one sodium pyruvate was added and to the second an equal volume of water. In two hours the flask containing the pyruvate gave 2.62 cc. of carbon dioxide, while the flask to which water had been added gave off only 0.7 cc., showing therefore the presence of this enzyme. Water extracts and banana juice were only slightly active with respect to this enzyme.

The authors desire to express their thanks to the United Fruit Company for their financial aid in this work.

Summary

An aqueous extract of crushed banana pulp contains material which activates the glycolytic action of yeast apo-zymase.

(5) Martland and Robison, *Biochem. J.*, **20**, 847 (1926).

Pressed juice from banana pulp also contains this activator.

The aqueous extract may be heated to boiling for a few minutes without loss of its activating property, but when evaporated to dryness on the steam-bath at P_H 6.4, it is rendered inert.

The glycolytic activator is more resistant to acid (P_H 1) than to alkali (P_H 9.4).

The activator passes through a collodion membrane on dialysis.

The effect of the activator, in the crude extract, on yeast apo-zymase is much less than that of yeast cozymase.

The effect of the activator on yeast apo-zymase is increased by small amounts of magnesium chloride, but hardly at all by acetic aldehyde.

The extract from the banana pulp also contained phosphatase and carboxylase.

NEW YORK CITY

RECEIVED AUGUST 13, 1932
PUBLISHED MARCH 7, 1933

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Studies on the Rearrangement of Tertiarybutylmethylcarbinol (Pinacolyl Alcohol). I

BY FRANK C. WHITMORE AND H. S. ROTHROCK[1]

In spite of the large amount of work on pinacolyl alcohol, its dehydration products and its halides, the subject is still confused. This is shown by the fact that the fourth edition of Beilstein's "Handbuch" gives the halides the tertiary structure, $(CH_3)_2CXCH(CH_3)_2$, corresponding to a complete rearrangement, while the supplement to the fourth edition gives the structure, $(CH_3)_3CCHXCH_3$.

In the present work the chloride has been prepared from pinacolyl alcohol and saturated aqueous hydrochloric acid at $-10°$. The product reacted with moist silver oxide to give the rearranged product dimethylisopropylcarbinol. A bromide was prepared from pinacolyl alcohol. This was studied by the freezing point method[2] and was found to consist of about 90% of the rearranged tertiary bromide, $(CH_3)_2CBrCH(CH_3)_2$. The composition of the impure bromide was not changed by heating for sixteen hours at 125°. Hydrolysis experiments[2,3] also indicated that the mixture was about 90% tertiary.

The dehydration of *tert.*-butylmethylcarbinol was studied. Attempts to dehydrate the boiling alcohol by means of traces of benzene sulfonic acid and iodine failed. Dehydration of the alcohol with oxalic acid gave tetra-

(1) Submitted in partial fulfilment of the requirements for the Ph.D. degree at the Pennsylvania State College. Most of this work was done at Northwestern University in 1928-1929.

(2) Whitmore and Rothrock, THIS JOURNAL, **54**, 3431 (1932).

(3) Delacre, *Bull. soc. chim.*, [4] **1**, 575, 978 (1907).