Occurrence and Some Characterization Studies of Invertase in the Green Plantain Fruit (*Musa parasidiaca*)

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INTRODUCTION

The development of off-flavors and the darkening of products prepared from green plantains have been problematic for some time in this Laboratory. These changes are suggestive of enzyme action. In order to understand the nature of these changes it was decided to investigate the enzyme systems present in the plantain fruit. As a first step in this study a determination of the presence and characteristics of invertase in the green plantain was undertaken.

MATERIALS AND METHODS

PRECIPITATION OF PROTEIN FRACTIONS

Green plantains of the Machete variety were peeled, cut into small pieces, and homogenized with water in a Waring Blendor. Three hundred milliliters of water were used for each 200 gm. of fruit. The suspension was filtered twice under vacuum to obtain a clear extract. The filtrate was then treated with ammonium sulfate until the desired percentage of saturation was reached. The salt was added slowly and with constant stirring. The solution was then left undisturbed overnight at 10° C. The next day it was centrifugated at 2,000 r.p.m. in a refrigerated centrifuge at 5° C. for 20 minutes. The precipitate was collected and dried under vacuum at 40° C. The supernatant liquor was then treated with additional ammonium sulfate until the next point of saturation was reached. The precipitate was separated and dried as before and the supernatant liquor treated with more ammonium sulfate. The procedure was repeated until the solution became 100-percent saturated with salt.

PURIFICATION OF PROTEIN IN FRACTION IA

The crude-protein material was resuspended in water and filtered. The filtrate was collected and coagulation was accomplished by the addition of $(NH_4)_2SO_4$ (100 gm./500 ml.). The salt was added slowly and with constant stirring. The precipitate was separated by centrifugation at 2,000 r.p.m.

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for 20 minutes. It was then resuspended in water and dialyzed through cellulose tubing against distilled water. The temperature was kept below 10° C. The procedure was carried out until a very faint test for $SO_4^=$ was obtained. The protein solution was then dried under vacuum at 40° C. and assayed for activity.

QUANTITATIVE DETERMINATION OF INVERTASE

Five-milliliter aliquots of 6.5-percent sucrose in acetic acid-sodium acetate buffer, pH 4.5, were pipetted into test tubes. The tubes were set in a constant temperature bath at 34° C. for 15 minutes. A 1-ml. aliquot of a dispersion of about 0.04 gm. of enzyme per milliliter of water was added to

TABLE 1.—*Invertase activity of different protein fractions extracted from green plantains*

Sample	Fraction[1]	Saturation with $(NH_4)_2SO_4$	Glucose produced	I.U.
		Percent	*Mg./2 ml.*	
I	A	100	0.0198	1.46
II	A	62	.0218	1.55
II	B	83	.0243	1.73
III	A	36	.0415	3.12
III	B	59	.0595	4.04
III	C	84	.0280	1.78
I	A^2	—	.273	17.9

[1] A^2 is the same as fraction I,A after first recrystallization.

each assay tube. After incubation time at 34° C. 2-ml. aliquots of the reaction mixtures were transferred to Folin-Wu tubes and assayed immediately for invert sugar by the colorimetric method of Nelson and Somogyi (*1*, *2*).[2] Controls were run for each set of samples using 5 ml. of 6.5-percent sucrose at pH 4.5 and adding 1 ml. of distilled water in place of the 1 ml. of enzyme solution.

RESULTS AND DISCUSSION

Protein fractions isolated from green plantains caused the inversion of sucrose in dilute solutions. Results are presented in table 1 in terms of glucose concentration and invertase units (I.U.). Invertase units are expressed as milligrams of glucose produced per gram of protein acting upon 6.5-percent sucrose, for 45 minutes at 34° C., and at a pH of 4.5.

[2] Italic numbers in parentheses refer to Literature Cited, p. 126.

Figure 1[3] illustrates the effect of enzyme concentration upon reaction rate. The reaction rate was proportional to enzyme concentration *i.e.* a linear function of enzyme concentration.

As a first approach to the investigation of the kinetics of plantain invertase the relation of enzyme activity to time of incubation was studied. As shown in figure 2 there is a linear increase in enzyme action with time of incubation. This means that a constant weight of the sugar present is split

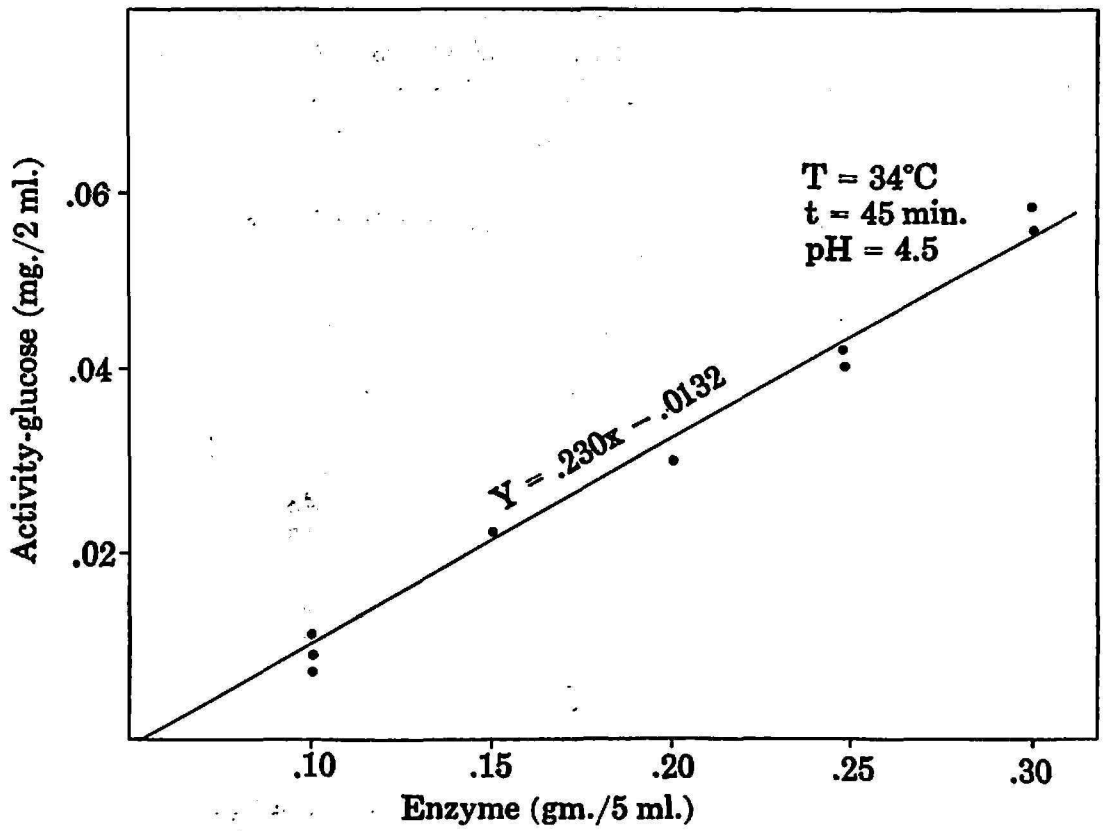

FIG. 1.—Effect of enzyme concentration on activity of green-plantain invertase.

per unit of time. The reaction, under the conditions used for this experiment, follows a zero-order reaction rate.

The effect of substrate concentration upon reaction rate is illustrated in figure 3. The plot shows a linear relation between activity and substrate concentration. As the number of sucrose molecules increases, plantain-invertase activity increases with a constant slope. As the concentration of sucrose exceeds a certain limit there is a decrease in the slope of the curve. The behavior of plantain invertase lends new support to one of the assump-

[3] The curves which appear in the illustrations with this paper were plotted following the equations obtained from statistical analysis of the data from a series of experiments. The equations proved to offer satisfactory explanations to the observed variations in y and all the constants were found to be statistically significant to the 1-percent level.

tions of enzyme kinetics which has received most experimental confirmation. This is the hypothesis of complex formation (*3*, *4*, *5*). This view assumes the formation of a complex between enzyme and substrate.

Such a complex is an activated molecule which decomposes into the products of the reaction and the original enzyme molecule. From this hypothesis one might expect a greater probability of complex formation with an increase in the number of collisions between enzyme and substrate.

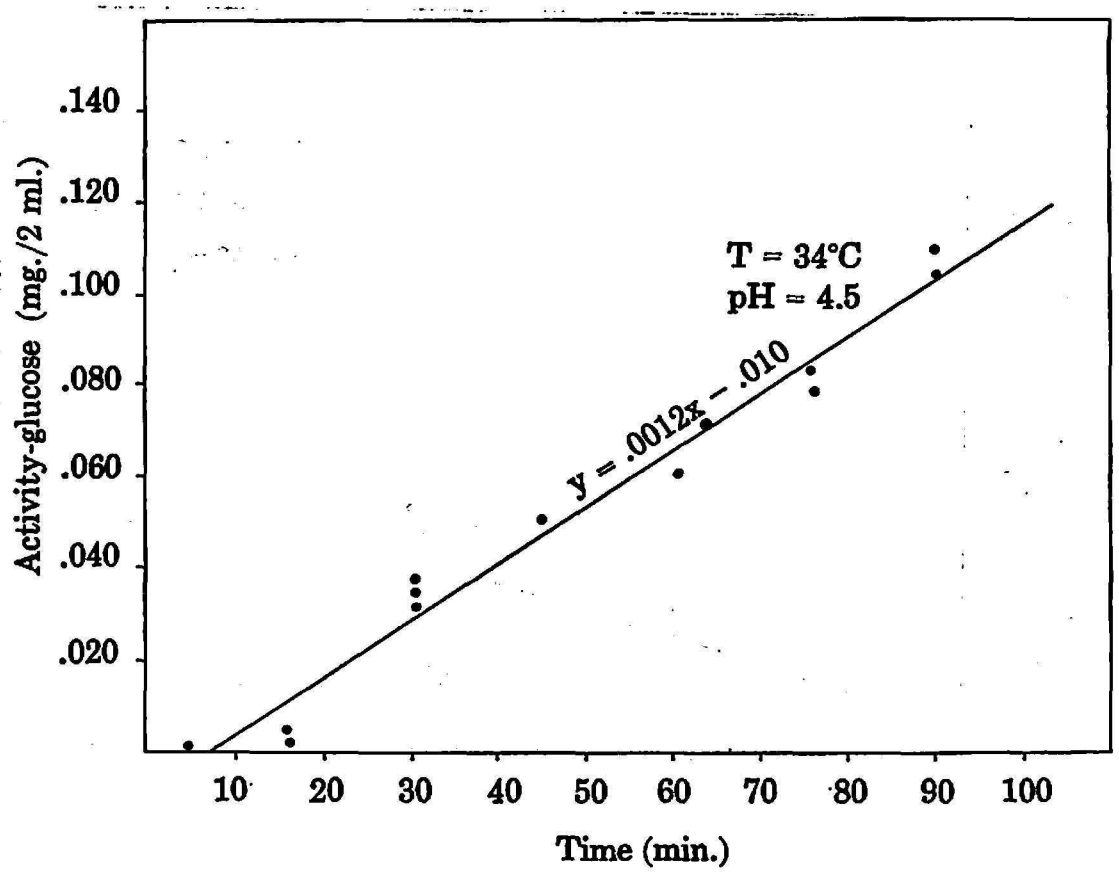

FIG. 2.—Relation of green-plantain invertase activity to time of incubation with a 6.5-percent sucrose solution.

This probability of collisions increases with an increase in the number of substrate molecules, thus causing an increase in reaction rate. As the substrate concentration exceeds a certain limit, the frequency of collisions looses importance as a factor in reaction velocity. This is shown in figure 3 by a decrease in slope as the concentration of sucrose reaches about 6 percent. This decrease in slope means that the addition of more substrate will not increase the velocity of the reaction by such a measurable degree as before.

Figure 3 indicates that the amount of sucrose hydrolyzed when the reaction is catalized by green-plantain invertase is proportional to the concentration of sucrose until it reaches a concentration of about 6 percent. This

indicates a first-order reaction rate up to a certain sucrose concentration. Then the rate becomes independent of the concentration of sucrose and the reaction behaves as one of zero order.

The influence of pH and temperature upon reaction rate is illustrated in figures 4 and 5, respectively. Parabolic relations were obtained for both. The curve representing the response of activity of invertase to pH is steeper than that of activity to temperature. This indicates that a small change in

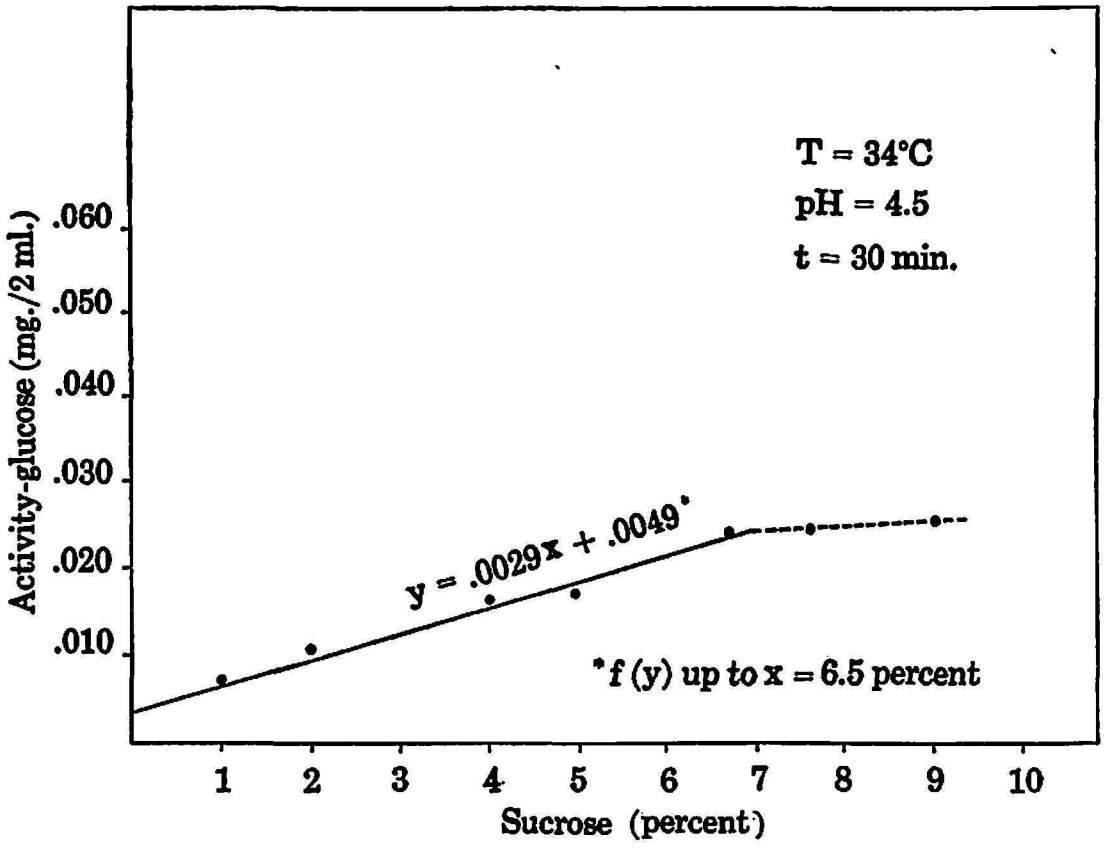

FIG. 3.—Effect of substrate concentration on reaction rate of sucrose inversion by green-plantain invertase.

the pH of the reacting medium will cause a greater variation in invertase activity than a small change in temperature.

Optimum conditions of pH and temperature were calculated from the equations obtained by statistical computations of the data for curves in figures 4 and 5. The first derivative, dy/dx, for each equation was calculated and the value of x when $dy/dx = 0$ was taken as the maximum for each curve. This gave an optimum pH of 4.15 and an optimum temperature of 44.4° C. for green-plantain invertase.

SUMMARY

The crude-protein fraction of green plantains was isolated and found to cause an inversion of sucrose solutions.

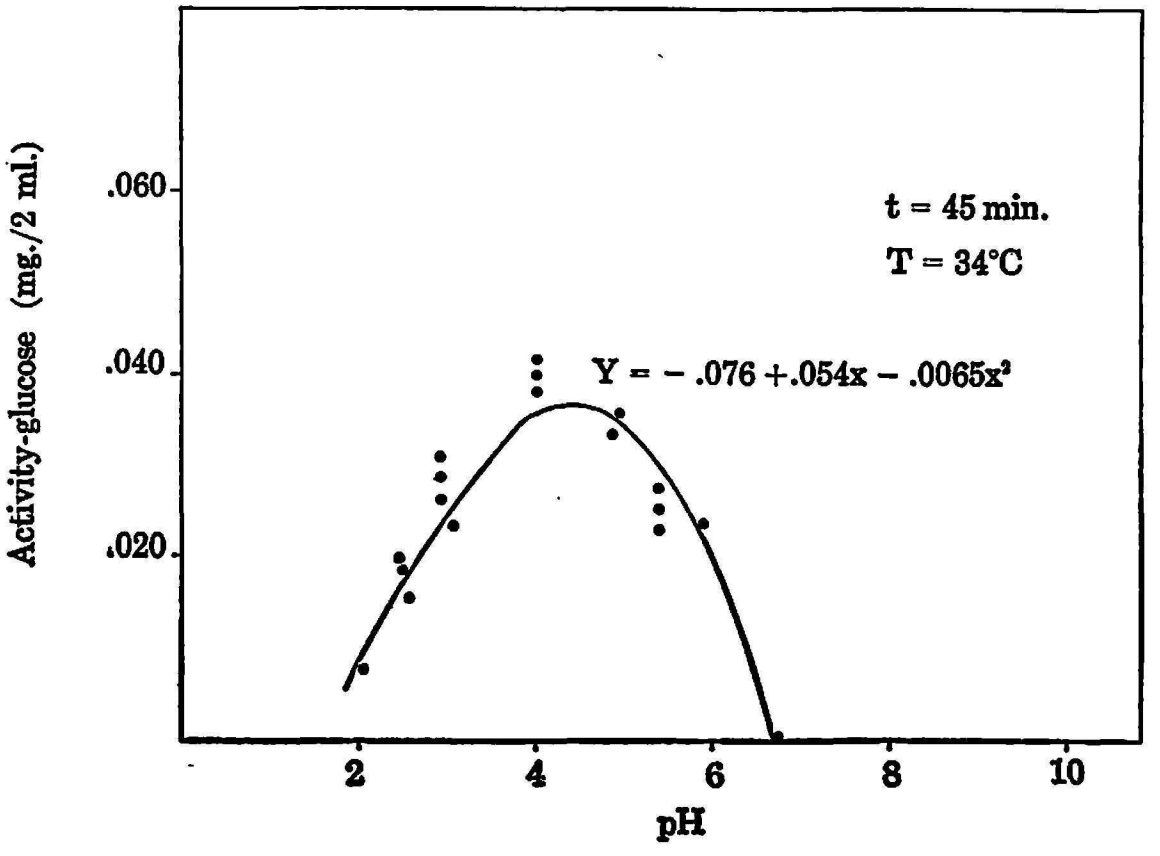

FIG. 4.—Influence of pH on activity of green-plantain invertase:

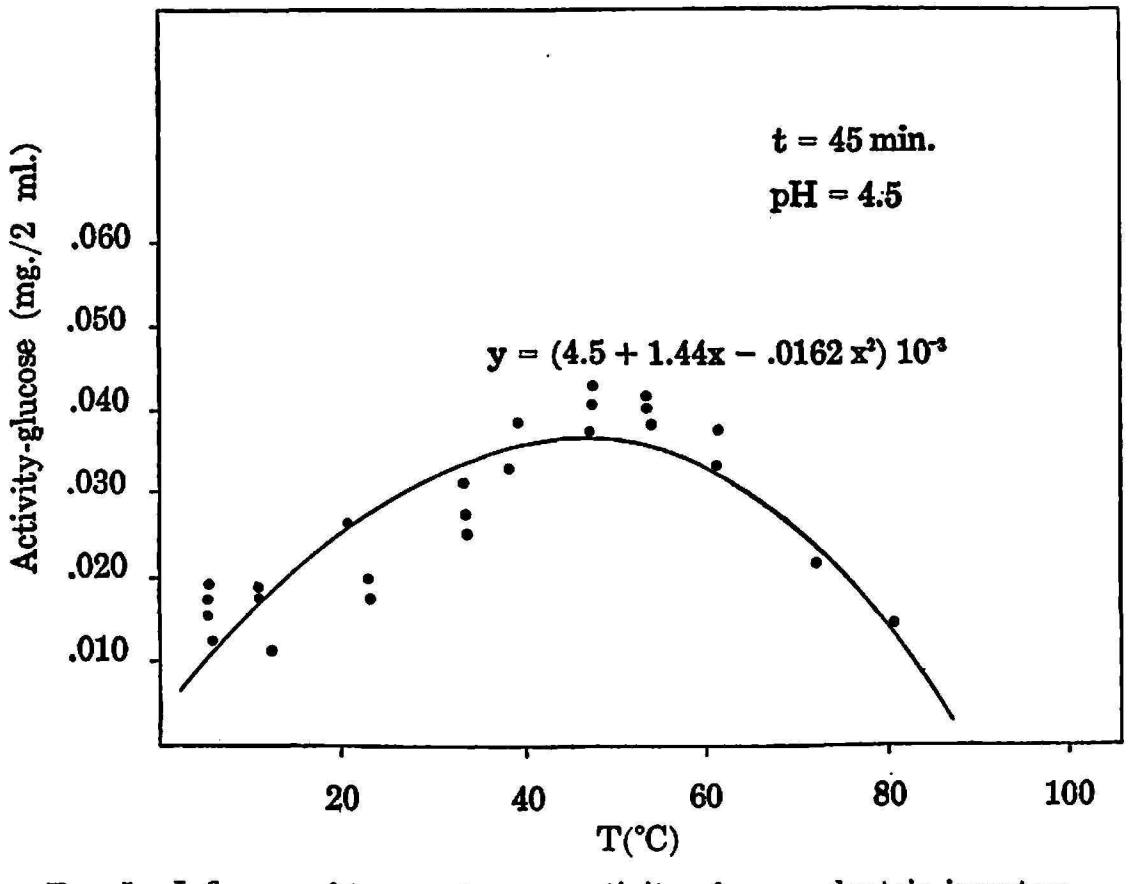

FIG. 5.—Influence of temperature on activity of green-plantain invertase.

The rate of inversion of sucrose by the invertase of the green plantain is proportional to the concentration of enzyme.

The inversion of sucrose, when catalyzed by green-plantain invertase, appears to follow a first-order reaction rate at low substrate concentrations (below 6 percent). As the concentration of sucrose exceeds 6 percent the rate of the reaction changes to zero order.

An optimum pH of 4.15 and an optimum temperature of 44.4° C. were obtained for the activity of green-plantain invertase.

RESUMEN

Se aisló la fracción proteica cruda del plátano verde y se encontró que ésta causaba la inversión de soluciones de sacarosa.

La razón de inversión de sacarosa por la invertasa del plátano verde es proporcional a la concentración de enzima.

La inversión de sacarosa, catalizada por la invertasa del plátano verde, parece seguir el comportamiento de una reacción de primer orden a concentraciones de substrato bajas (bajo 6 por ciento). A medida que la concentración de sacarosa excede el 6 por ciento, la reacción se comporta como una de orden cero.

Se encontró un pH óptimo de 4.15 y una temperatura óptima de 44.4° C. para la invertasa extraída del plátano verde.

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