ON BANANA AMYLASE.

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1. Introduction.

The essential change occurring during the ripening of the banana (*Musa sapientum*, Linn.), is the conversion of starch into soluble, simpler carbohydrates. The mechanism of starch degradation has formed the subject of several investigators, many of whom have incriminated amyloclastic enzymes as the agencies responsible for such changes; unfortunately the results obtained so far have been highly conflicting and the question whether or not such agencies are present, still remains obscure.

The earliest work of Tellarico (1908) and Bailey (1912) on banana amylases has been re-examined by Falk and McGuire (1921) who, by the use of more quantitative methods, failed to demonstrate any "definite and marked" amylase action in their banana pulp preparations or extracts. The negative results according to them are attributable to the presence of the substances in the banana, "which when brought into close contact, as by grinding with the enzyme material, inhibit the action...It is also possible that the ripening process or the breaking down of starch is not merely an amyloclastic action as this is commonly understood but involves a simultaneous or preliminary oxidation reaction." Ranganathan (1928) and subsequently Desikachar (1931) failed to detect any amylase in the ripening bananas, thus confirming the results of Falk and McGuire.

Evidence as to the existence of an amylase was, however, indicated by an interesting observation of Ranganathan. Unripe bananas, cut into slices and covered with toluene, showed, on incubation, increasing concentrations of soluble sugars, while a similar group of control slices covered with toluene but heated in a steam oven for 30 min. did not yield any such increase in the sugars, thus indicating the presence of a thermolabile agent responsible for the saccharification of the reserve starch. Attention should be drawn to the positive results of Bailey (1912) who in the course of his experiments on the digestion of starch by banana extracts, obtained a progressive degradation of starch as indicated by the iodine colorations,

blue to red, a significant result not fully appreciated by the subsequent investigators. Bailey's conclusions have been confirmed by the findings of Uchihara (1920) who has obtained positive tests for amylase in banana by an entirely different method. In view of the controversial nature of the problem it appeared desirable to re-examine the question, particular attention being paid to the methods of enzyme extraction rather than to those of estimation of the amyloclastic activity.

2. *Experimental.*

A study of the rate of saccharification of starch in the ripening fruit, shows that the change proceeds most rapidly during the period when it is passing from green to yellowish green. The material used for our experiments was at the yellowish-green stage of ripeness.

Cross sections of the fruit (1) for tannins and (2) for starch during varying stages of ripeness were examined histochemically. It was expected that the location of tannins *in situ* would be helpful in eliminating the tannin-bearing portions of the tissue, and thus secure an enzyme extract or powder free from tannin which is known to inhibit amyloclastic activity. The tannin content of pulp in the ripening fruit has been estimated to be 1·52—1·66 per cent. (Ranganathan, 1928). Staining the tissue with a dilute solution of ferric chloride (1 part of $FeCl_3$ in 250) reveals that the tannin is confined mainly to the periphery and the three bands (Fig. 1) radiating from the centre. By staining with iodine, it can be demonstrated that the section stains a deep bluish black in the unripe stage. As the ripening proceeds starch disappears largely but a small quantity has been found to persist in the middle and in the region round the tannin-containing bands, already referred to, thus pointing to the inhibitory action of tannin on the hydrolysis of starch. The course of disappearance of starch in ripening bananas had been previously demonstrated by Hartshorn (1931). The histological examinations further reveal that the disappearance of starch starts from the periphery and gradually extends towards the core. These observations suggest the probability that the peel is the seat of the enzyme. Osmotic pressure measurements made by Stratton and Loesecke (1931) have shown that during banana ripening the osmotic pressure of the pulp which equals that of the peel in the green condition rapidly increases, so that a streaming of the liquid takes place from the peel to the pulp.

These preliminary studies emphasised (1) the need for differentiating and examining the pulp and the peel separately, and (2) the need for effectively removing inhibitory tannins during the extraction.

3. Methods of Extraction.

Mature, ripening bananas were separated into pulp and peel. Great difficulty was experienced in obtaining the peel entirely free from pulp and our peel preparations therefore represent a slight admixture of the outer layers of the pulp. 10 parts by weight of the green tissue mixed with 2 parts of hide powder was comminuted in a mortar and ground to a paste after adding a sufficient quantity of acid-washed sand. The paste was then treated with 100 parts of 80 per cent. glycerine and the mixture centrifuged at 3,000 R.P.M. to obtain the clear extract.

For obtaining dry preparations of the material the tissues after rapid mincing were treated with successive batches of absolute acetone to obtain a dry material. Contact with acetone appears to be deleterious to the enzyme and should therefore be minimised. The dry material thus obtained is found to be free from tannins and most of the soluble sugars. Whole banana powders were also prepared in a similar manner, the material being grated and immediately dropped into acetone. 20 gms. of dry powder were extracted with 100 c.c. of 50 per cent. glycerine, shaken for 4—5 hrs., centrifuged, yielding a clear centrifugate.

4. Estimation of amyloclastic activity.

The amyloclastic activity was determined by employing a reaction mixture consisting of 20 c.c. of 2 per cent. soluble or banana starch, 20 c.c. of the extract and 1 c.c. of toluene. The reactions were carried out at 40°C. Two controls were found necessary; one which may be termed a *live* control consisting of 20 c.c. of the extract, and 20 c.c. of water, in order to correct for the reducing sugars obtained by the action of invertase on cane sugar that may be present in the extract, and the other control consisting of the more usual boiled extract treated with an equal volume of starch. From time to time, a small quantity of the reaction mixture was withdrawn from the flask and tested with iodine.

After incubation for 24 hrs., 20 c.c. of the reaction mixture was pipetted out into 100 c.c. of 95 per cent. alcohol, filtered, precipitate repeatedly washed with alcohol, filtrate and washings evaporated on a water bath to a syrup, taken up with water and made up to 50 c.c. The reducing sugars in 20 c.c. aliquots were estimated by Bertrand's method.

5. *Results.*

TABLE I.

Reaction mixture : 20 c.c. of extract + 20 c.c. of soluble starch solution (Mgms. of maltose produced after 24 hrs. incubation at 40°C. Calculated to 100 c.c. of reaction mixture).

Variety	Extracts of green tissues			Variety	Extracts of dry powders		
	Peel	Pulp	Peel + Pulp		Peel	Pulp	Peel + Pulp
* *Elakki*, 27-1-33 .	96	..	110	**Elakki*, 22-3-33 ..	132	..	156
,, 9-2-33 ..	128	slight	162	,, 28-3-33 ..	179	..	186
,, 21-2-33 ..	78	..	108	**Rasabale* 4-3-33..	128	..	147
* *Puttabale*, 28-2-33.	46	..	73	,, ..	110	..	128

Influence of tannin on Amylase activity.—That the tannins isolated from banana inhibit the amylase activity of the extract, is clear from the following table. 20 and 10 c.c. aliquots of a 50 per cent. glycerine extract of whole-banana powder were incubated with varying quantities of tannin extracts (prepared from the banana peel by extraction with ethyl acetate) and 20 c.c. of 2 per cent. soluble starch. After 20 hours, the experimentals as well as the blanks, were treated with alcohol, the residues from the filtrates were taken up with water diluted suitably and the reducing substances estimated in aliquots by Bertrand's method.

TABLE II.

	20 c.c. starch + 20 c.c. enzyme extract	20 c.c. starch + 10 c.c. enzyme extract + 10c.c. water	20 c.c. starch + 20 c.c. enzyme extract + 1 c.c. tannin	20 c.c. starch + 20 c.c. enzyme extract + 2·5c.c. tannin solution
c.c. of Permanganate N/10 ..	3·8	2·1	0·1	..
Coloration with Iodine ..	Red	Violet	Blue	Blue

6. *Discussion.*

An examination of the results (Table I) indicates that there is a definite saccharification of starch with peel tissue whether employed as extract or as powder. A study of the iodine coloration which was also followed with the

* Local vernacular names for different varieties of bananas.

progress of hydrolysis showed that the coloration gradually changes from blue to a pure brick red which does not entirely disappear even after prolonged action for 48 hours. It should be mentioned that the coloration which develops on addition of iodine is evanescent, but persists long enough to be observed. Since such a rapid disappearance is not noticeable when dialysed extracts are employed, it is clear that the phenomenon is attributable to the presence of some strongly reducing substances in the crude extract.

Experiments with extracts of peel and pulp individually and also in mixture establish that (1) the amylase is definitely present in the peel extract, (2) that the pulp extracts exhibit little or no activity, and (3) that a mixture of the extracts of peel and pulp, invariably shows a greater activity than either of them when employed individually, thus pointing definitely to the existence of activators in the pulp extract, whose nature remains to be determined.

The failure of previous investigators to demonstrate the enzyme activity is due to the circumstance that most of them looked for the enzyme in the isolated pulp; further, no attention was paid to the effective elimination of the inhibitory tannins during the course of the preparation of the enzyme extract, a precaution which has been shown to be essential in securing active preparations of amylase. The decisive influence of tannin on the course of amyloclastic activity is strikingly brought out by the histochemical examination of the cross-sections of the ripening banana (Fig. 1). The persistence of starch in regions of high tannin concentration even in the advanced stages of ripening and the disappearance of starch in regions where tannins disappear or are absent, point to the existence of a fine mechanism which controls the process of ripening in starchy fruits.

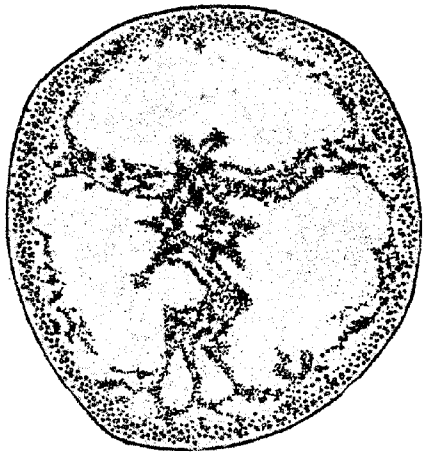

FIG. 1.
Cross section showing the tannin-bearing regions.

7. *Summary.*

(1) Tannin-free extracts of the banana peel (possibly admixed with the outer layers of the pulp) possess marked amyloclastic activity as revealed by the iodine and saccharogenic tests.

(2) Evidence has been obtained to show that the pulp extracts while themselves being incapable of hydrolysing starch, contain activators which enhance the activity of peel extracts.

(3) Histochemical examination of ripening fruits reveals that ripening is controlled by the presence or disappearance of tannins.

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