II.—CARBOHYDRATE CHANGES DURING THE RIPENING OF PLANTAINS.

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The following note contains an account of some preliminary experiments carried out on the ripening of the plantain. The work is far from complete but as it has had to be discontinued it is desired to place the results on record.

The chemical changes which starch-containing fruits undergo during their ripening processes have been the subject of numerous studies. The plantain is a typical example of such fruits and is particularly suitable for investigation because of the possibility of readily obtaining the fruit at different stages of ripeness, the quickness with which it ripens and the wide changes from starch and other higher carbohydrates to the simpler substances glucose, invert sugar and sucrose accompanying the ripening. By analogy with the various changes in plants brought about by specific enzymes we might expect the presence of a starch-splitting enzyme during one or more stages at the period of ripening or, failing this, it should be possible to ascertain by what agency the changes are brought about. Bailey (*J. Biol. Chem.*, 1906, I, 355) made a careful investigation of the conditions under which the ripening proceeds and showed that in absence of oxygen the change does not take place. Tellarico (*Amer. Chem. Abs.*, 1908, II, 2105) and Jahkel both considered the change to be brought about by means of amylase. Bailey (*J. Amer. Chem. Soc.*, 1912, **34**, 1706) concluded from his experiments that 'amyloclastic action is slight in the very green fruit and increases as maturation proceeds. Starch hydrolysing power is retained by the powdered tissue.' K. G. Falk and G. Meguire (*J. Gen. Physiol.*, 1921, **3**, 595) did not find evidence of the presence of a starch-splitting enzyme. They conclude 'it is possible to imagine a cellular structure of such a nature that the enzyme material and an 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 structure.'

EXPERIMENTAL.

Each fruit was removed from a bunch of unripe plantains and the cut end at once seared with a hot glass rod and sealed with paraffin. The fruits were kept for a number of days and every day one or more taken for analysis. Sampling for the estimations was done according to the A. O. A. C. methods and the sugar estimation made by Bertrand's process.

Analysis of plantains at different stages of ripeness.

Co-efficient of ripeness	Acidity c.c. N/10 alkali for 10 gm. pulp	Reducing sugars per cent.	Total sugars per cent.	Total carbohydrates per cent.
2·5	5·4	2·84	4·29	18·77
2·7	9·9	4·44	11·20	18·99
4·06	9·8	7·56	14·28	18·79
4·5	9·9	10·17	17·81	18·76
5·62	10·2	12·46	17·43	18·69
6·23	9·4	12·69	18·23	18·60

The term 'co-efficient of ripeness' is a convenient term used by previous investigators and denotes the ratio of the weight of the pulp to the weight of the peel. As the ripening proceeds there is a marked increase in the ratio. Clearly there is a definite and systematic increase in the amount of reducing and non-reducing sugars, but the total carbohydrate content remains almost constant. The point of interest is the mechanism of sugar-production.

Examination for amylase.—Transformation of starch into sugars is most marked when plantains are just passing from green to yellowish green, and such were used for the tests. The very green plantain was also tested. The fruit was pulped with water, dilute alcohol (20 per cent.) or glycerol, in every case a few drops of toluene being added to inhibit bacterial action. The mixture was allowed to stand with frequent shaking for at least four hours. After filtration through muslin the extract and the residue (air-dried) were tested for enzymic activity. The substrate used was a 1 per cent. solution of Lintner's soluble starch (B.D.H.). In no case was there any evidence of amyloclastic activity.

Autolysis trials.—The pulp was well mashed with a little toluene, made up to a known volume and allowed to stand for 24 hours. A second sample of pulp was prepared similarly, but was then kept in a boiling water bath for some minutes; the volume was then adjusted and the second sample incubated under the same conditions as the first for which it served as a control. After 24 hours the amount of sugar in both preparations was the same, thus giving negative evidence of diastatic action.

Ripening in absence of oxygen.—Bailey's experiments to investigate the effect of oxygen-deficiency on the ripening process were repeated. Unripe plantains from the same bunch were kept (1) exposed

to air, (2) immersed in liquid paraffin, or (3) corked up in a small air-tight tube. After a few days the percentage of sugar in each sample was estimated and confirmed the conclusion that oxygen is essential to the ripening process. Plantains in two stages of ripening were used.

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Total sugars in unripe pulp	3·8	7·3
1. Exposed to air for 43 hours	6·8	15·5
2. In air-tight tube	5·1	13·7
3. In paraffin...	3·9	7·5

The colour of the peel also indicated that ripening had not occurred when oxygen was excluded.

Tannin in plantains.—There is the possibility of tannin or some other material which may occur in the plantain inhibiting the action of amylase in the extracts obtained when the cells are ruptured as in the above experiments. The tannins in plantains just beginning to ripen were estimated by the gelatine precipitation method (A.O.A.C.). The percentage calculated on the total dry pulp varied from 1·52 to 1·66 per cent.

The tannins were removed from the plantain pulp by extraction with 95 per cent. alcohol for four hours in the shaking machine. An aqueous extract of the residue thus freed from tannins was tested for amylase but again the results were negative.

Examination for other inhibitors.—There may be present in the pulp some inhibiting or paralysing substances not necessarily tannins. The extract of the plantain pulp was therefore added to preparations of barley amylase and taka-diastase and the effect on the amyloclastic activity studied. The results tabulated below show that the malt diastase acts on the substrate (1 per cent. starch) quite as vigorously in the presence of plantain extract as in its absence. Sugars were estimated after 24 hours' incubation at 37°.

Amount of sugars represented by c.c. of standard potassium permanganate used in the estimation.

Source	Period of incubation	Control without plantain extract : sugar formed	Diastase and plantain extract : sugar formed
Taka-diastase	30 mins.	5·0	5·0
	18 hrs.	10·8	10·9
Barley malt diastase ...	40 ,,	13·3	13·4
	24 ,,	14·0	13·9
	24 ,,	12·4	12·6
	24 ,,	18·9	18·7

Effect of heat.—If the change is due to enzymes a strongly heated sample should fail to ripen owing to destruction of enzyme. An unripe plantain was therefore cut into thin slices arranged in two groups in Petri dishes. The pieces were covered with toluene for a few minutes and then drained. One set was heated in steam for half-an-hour when both were allowed to stand for two days. The pulp from each set was then dried and the sugars estimated. The heated pulp contained 2 to 3 per cent. less sugars than the unheated sample (the percentages being calculated on the weight of air-dry powder), but in both there was an increase of sugar-content. Thus the heating has not completely arrested the change. As the heating may not have been sufficiently prolonged to destroy all the enzymes of the cell, the experiment is somewhat inconclusive but lends some support to the theory that the change is enzymic.

SUMMARY.

1. Extracts prepared from ripening plantains by various methods have all failed to exhibit diastatic activity.

2. It has been shown that tannin-free pulp also does not show amyloclastic action and that the pulp material does not inhibit or paralyse malt diastase, so that the negative results obtained are in all probability not due to the presence of inhibiting agents.

3. The complete dependence of the ripening process on the presence of oxygen does not preclude the existence of enzymic actions as has been shown by the work of Oparin.

4. The experiments here described therefore generally confirm those of Falk and Mcquire in contrast to the results of Tellarico and of Bailey.

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