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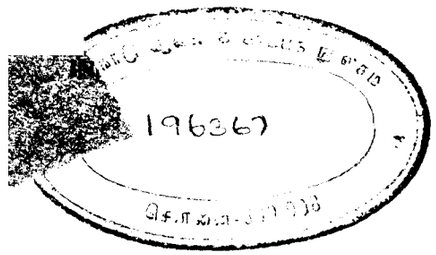


I.—ESTIMATION OF IODINE IN SOILS.

By Roland V. Norris and D. A. Rama Rao.

Recent work has tended to emphasise the importance of iodine both in the animal and vegetable worlds. This element is an essential constituent of thyroxin the active principle of the thyroid secretion; and the almost universal occurrence of a thyroid mechanism amongst the vertebrates points to the conclusion that there is a definite iodine metabolism in these forms which is an essential part of their vital processes (cf. Swingle, *J. Gen. Physiol.*, 1920, **2**, 161).

A deficiency of iodine in the thyroid has been considered by many workers to be at any rate a predisposing cause of goitre and this deficiency has been attributed to a lack of iodine in the soils from which the food and water supplies of persons so affected have been derived (Bruce, *Pres. Address, British Association*, 1924).

In the vegetable kingdom also it is believed that iodine plays an important part in the synthetic processes of the living cell (Stoklasa, *Z. Angew. Chemie*, 1927, **40**, 20). The detection and estimation of small quantities of iodine in biological materials, foodstuffs, soils, etc., is therefore a process of much importance and various methods have been suggested in recent years. Of these the more important are those described by Kendall (*J. Biol. Chem.*, 1914, **19**, 251), Kelly and Husband (*Biochem. J.*, 1924, **18**, 951), Pickworth (*Biochem. J.*, 1925, **19**, 769), McClendon (*J. Biol. Chem.*, 1924, **60**, 289), Hercus, Benson and Carter (*J. Hygiene*, 1924, **24**, 321 and Leitch and Henderson *Biochem. J.*, 1926, **20**, 1003).

Kendall's method as modified by Kelly and Husband consists in fusing the material with sodium hydroxide, the destruction of the organic matter being assisted by the addition of potassium nitrate. The iodine is then extracted with water, oxidised to iodate by means of bromine water and the iodine liberated on addition of potassium iodide titrated with *N*/200 sodium thiosulphate. In Pickworth's method the iodide extracted after fusion of the material is oxidised by means of potassium permanganate, the excess of this reagent being removed by animal charcoal. The iodate is treated with potassium iodide and the liberated iodine titrated as above with thiosulphate.

McClendon mixes the material with lime and carries out a combustion in a stream of oxygen, any iodine volatilised being absorbed in alkali. The absorption liquid, as also the extract of the

combustion residue, is acidified, any iodate present reduced to iodide by the addition of a trace of arsenious acid and the iodine liberated by means of sodium nitrite. The free iodine is then taken up in carbon tetrachloride and estimated colorimetrically.

Fellenberg, who at the suggestion of the Swiss Goitre Commission has made an extended study of the methods of iodine estimation, ignited the material with potassium hydroxide and extracted the ash with alcohol. The iodine liberated by the addition of sulphuric acid and potassium nitrite was taken up in chloroform and estimated colorimetrically. Fellenberg's method has been modified by Leitch and Henderson, who prefer the titration method to the colorimetric method; these authors also utilise the iodate-potassium iodide reaction by which the amount of iodine to be titrated is increased six times. The methods outlined above have been utilised chiefly for biological materials and foodstuffs and but little work is on record relating to the estimation of iodine in soils. In the latter case the difficulties are much increased as, owing to the large amount of inert material present, it is by no means easy to bring about a satisfactory destruction of the organic matter without loss of iodine. The largest series of soil analyses is that carried out by Hercus, Benson and Carter in New Zealand (*J. Hygiene*, 1924, **24**, 321). These authors give the results obtained with some 400 different soil samples, the iodine content of which varied between nil and 700 parts per 10 million parts of soil.

The method employed was to soak the soil with 8 per cent. alkali and ignite it at a low red heat for about 15 minutes; the ignited material was extracted with boiling water, the extract neutralised. the iodide oxidised to free iodine by nitro-sulphuric acid and the free iodine taken up in carbon disulphide and titrated with $N/1270$ thiosulphate. Figures for control soils with known amounts of added iodine are not quoted. The authors however realised that the extraction of the soluble iodine from the soil by this method was not always complete and state (*loc. cit.*, 331) 'the method here described yields figures approximately *proportional* to the amount of soluble iodine in by far the greater number of the soils analysed.'

EXPERIMENTAL.

The Carter method was first tested on a sample of local soil. It was anticipated that difficulties might arise owing to the considerable amount of iron present in this soil as evidence has previously been brought forward indicating that loss of iodine may occur in such circumstances. This point therefore was examined first. To eliminate any difficulties that might arise from the presence of organic matter the soils used in this experiment were first ignited with ammonium nitrate. A known weight of the soil was then treated with a dilute solution of

potassium iodide of such strength that the sample when dried contained 1,000 parts iodine per 10 millions parts of soil. A second sample was prepared in the same way containing half this quantity of iodine. A third sample of soil without added iodine was used as a control. The iodine in all three samples was then estimated by Carter's method with the following results:—

...	A	B	C
Parts iodine added per 10 million parts soil	1,000	500	0
Iodine titration	10·0	5·5	0
	8·0	3·9	0
	6·5	4·0	0
Theoretical titration figure	25·0	12·5	0

That the unsatisfactory results obtained were probably due to the iron content of the soil is indicated by the following experiments carried out with pure quartz sand and similar sand impregnated with oxide of iron.

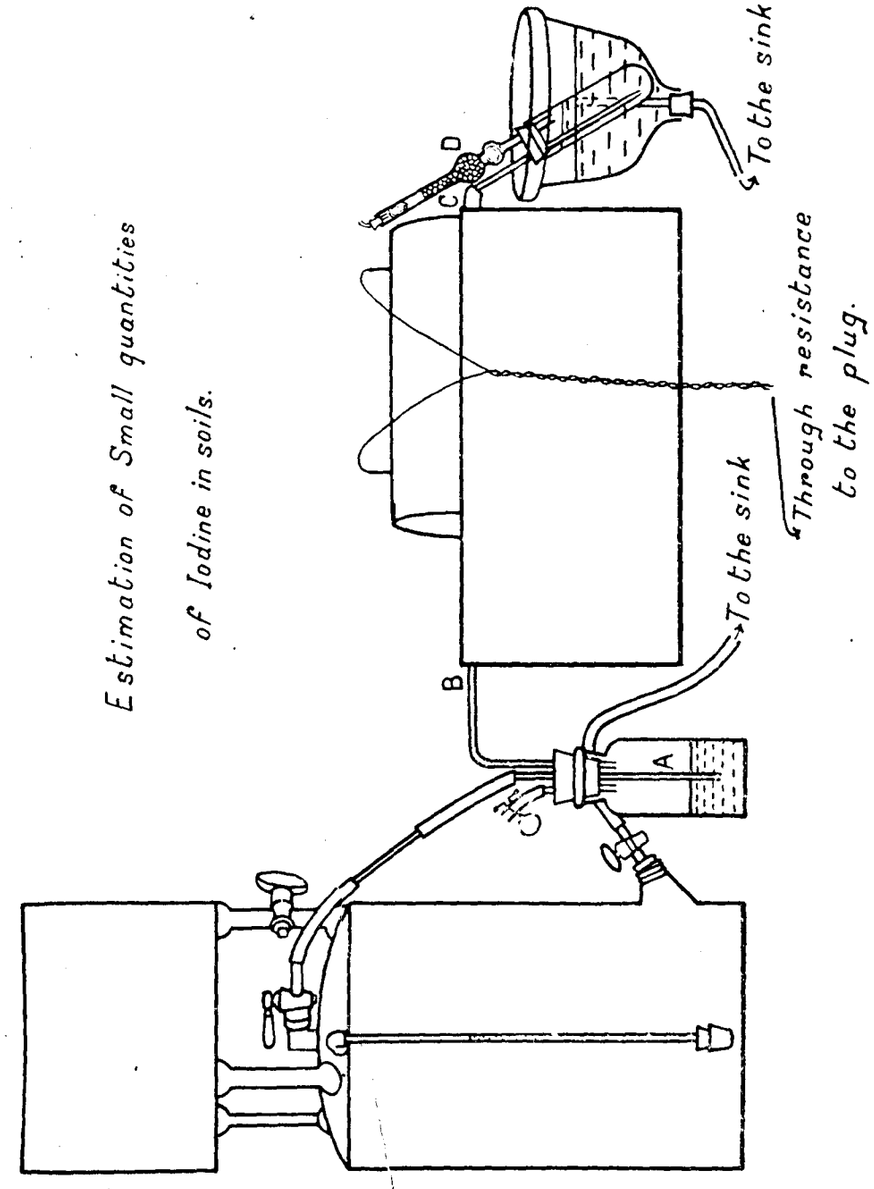
The sand used was first boiled with commercial hydrochloric acid for 15 minutes, well washed and dried. Potassium iodide solution was then added in such quantity as to give samples with an iodine content of 1,000 and 500 parts per 10 million of sand respectively. A second portion of the sand was treated with a solution of iron pernitrate, dried and strongly ignited. In this way the sand was impregnated with iron oxide, the content of iron being 1 per cent. and 3 per cent. respectively in the two samples prepared. Potassium iodide was then added so as to give a sample containing 1,000 parts iodine per 10 million of sand. All the samples were then analysed by Carter's method with the following results:—

...	Samples free from iron		Samples containing oxide of iron	
	A	B	C. 1 per cent. iron	D. 3 per cent. iron
Parts iodine added per 10 million sand	1,000	500	1000	1000
Titration figures	10·0	4·9	5·55	5·50
	9·9	4·9	5·65	5·55
Theoretical titration value ...	10·0	5·0	10 0	10·0

It will be seen that while the sand alone has given correct results, in the presence of iron the result is only 56 per cent. of the correct figure. The loss may be due to either volatilisation of free iodine in the ignition or to a failure in the extraction process in the one case. To facilitate the extraction it was considered advisable to raise the temperature of ignition ; to guard against loss of iodine by volatilisation the ignition was carried out in a combustion tube and any volatile products collected in caustic alkali. In this respect the process resembled McClendon's method. It was soon realised however that the extraction process could be dispensed with altogether if the soil were ignited at a sufficiently high temperature without the addition of alkali ; extraction being a troublesome and slow process this procedure has obvious advantages. The whole of the iodine will now be in the absorption liquid and can be estimated in this solution by means of any of the methods already described. Theoretically the oxidation of the iodine to iodate and treatment of this with excess of potassium iodide seems advantageous as by this method six times as much iodine is obtained for titration as is actually present in the soil. In practice however we have found this procedure unsatisfactory. During combustion of the soil other compounds capable of liberating iodine from potassium iodide appear to be formed and the results are consequently high. We have found it preferable to estimate the iodine by titration with $N/1270$ thiosulphate after extraction with carbon tetrachloride. This solvent has been found to give more reliable results than carbon disulphide which was used by Carter. The method finally adopted is as follows.

The combustion of the air-dried soil is carried out in a nickel boat about 10 inches long in a silica combustion tube in a stream of oxygen. For soils rich in iodine 5 gms. can be used, the quantity being increased if the figure is likely to be low. The combustion tube is heated to about 850° in a simple type of electric furnace. One end of the tube is drawn out to a fine point which is bent over and passes directly into the absorption vessel containing a small quantity of 10 per cent. sodium hydroxide. We have not found it necessary to use any cooling coil as suggested by McClendon. The combustion is continued for one hour by which time the whole of the iodine present in the sample will have been driven over into the alkaline solution. The latter, together with the washings of the absorption vessel, is then transferred to a 100 c.c. stoppered bottle and neutralised by the careful addition of sulphuric acid ; 2 c.c. of carbon tetrachloride are then added and the iodine liberated from the iodide by the addition of 3–5 drops of nitro-sulphuric acid, prepared by heating concentrated nitric acid with starch and absorbing the nitric oxide fumes in sulphuric acid. After vigorous shaking the carbon tetrachloride containing the iodine is separated, washed twice with a very small quantity of water and freed from acid by adding 2 c.c. of sodium acetate solution. The iodine is then

Estimation of Small quantities of Iodine in soils.



titrated with $N/1270$ sodium thiosulphate. If necessary a second extraction with carbon tetrachloride may be made. The thiosulphate solution is kept in the dark and standardised at frequent intervals.

We have found this method to be simple and reliable. The coloration produced by the presence of 0·005 mgm. iodine in 2 c.c. of carbon tetrachloride is perfectly perceptible. In 25 gms. of soil this would correspond to 2 parts of iodine per 10 million of soil. This quantity of iodine, however, only requires one drop of $N/1270$ thiosulphate and cannot with certainty be detected in a soil analysis. Using the above quantity of soil, however, 5 parts of iodine per 10 million can be detected and greater amounts estimated.

The results obtained with a number of Indian soils examined varied from nil to 400 parts per 10 million. Details will be found in another publication (McCarrison, Newcomb, Viswanath and Norris, *Ind. Jour. Med. Res.*, 1927, **15**, 207).

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II.—CARBOHYDRATE CHANGES DURING THE RIPENING OF PLANTAINS.

By S. Ranganathan (Junior).

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, **1**, 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.

	I	II
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
	40 ,,	13·3	13·4
Barley malt diastase ...	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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