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used shaken from the roots of garden-plants, which are no Papilionaceae, the result is fairly the same; perhaps the number of the above mentioned oxidising forms is more numerous, but this is still doubtful.

Otherwise, however, is the result when the soil is examined which adheres to the roots of clover, pease, and beans when these plants are cautiously dug up. When the soil adhering to such roots is rubbed fine and after dilution in water sown on a malate plate we find, after a period of 2 days at 30° C., first that the said oxidising colonies have very abundantly developed. But, besides, among these colonies much larger ones are distributed, which oxidise much more vigorously and prove to belong to *Azotobacter*, which shows that a distinct relation exists between the distribution of this genus and the said Papilionaceae. Whether this relation will appear to be universal and what may be its signification, further experiments have to decide.

Chemistry. — *"Rapid change in composition of some tropical fruits during their ripening."* By H. C. PRINSEN GEERLIGS.

(Communicated in the meeting of May 30, 1907).

Some tropical fruits which as a rule are gathered in a green and immature state and allowed to ripen afterwards, accomplish this ripening process so rapidly that within a few days they become tender, well-flavoured and palatable, thus offering a good opportunity for studying the still somewhat mysterious problem of the after-ripening of fruits.

I. Phenomena during after-ripening.

a. Banana (Musa).

As a rule the bunches of bananas, which contain fruits in various stages of maturity, are cut from the plant as a whole when all the fruits are still green and are hung up to ripen. At the moment when the bunch is cut none of the bananas are fit for food; they are hard, tasteless and flavourless, the skin is thick, contains much latex and tannin and adheres to the fleshy part. After a few days the skin becomes thin and yellow and can easily be detached, whilst the edible matter is now tender, sweet and well-flavoured. A couple of days afterwards the fruit is unpalatable again owing to overripeness and decay which change it into a soft mass.

This after-ripening is accompanied by a considerable loss of weight as is shown by the following figures.

20 bananas broken from the bunch in a green state were placed in a relatively cool spot (28° C.) and weighed daily.

The average weight per fruit was:

after	0	1	2	3	4	5	6	7	days
	145	143	142.5	142	141	139	138	137	grammes.

Further, 10 green bananas of another variety were placed under a glass bell jar into which a current of air free from carbonic acid was introduced. The air leaving the bell jar was made to pass through a drying apparatus and a Liebig potash bulb, which latter was daily weighed.

The fruits weighed originally	502.5	grammes
and after 4 days only . .	487.0	,,
therefore lost in weight . .	15.5	grammes

The weight of the potash bulb increased

the first day by	0.065	grammes
the second day by	1.455	,,
the third day by	0.540	,,
the fourth day by	0.240	,,
Total increase	2.300	grammes

So that the fruits gave off 2.3 grammes or 0.44 % of carbonic acid in four days.

The chemical changes taking place during the after-ripening process were now studied. Each day a banana was broken off from a green unripe bunch of the fruit and when doing this care was taken to select the specimen from the same row of the bunch from which the previous one had been taken and thus to obtain samples of the same initial ripeness. The fruit was first peeled and rubbed to pulp in a mortar. I determined the amount of moisture by drying 10 grammes to a constant weight. Next 100 grammes of the pulp were extracted with alcohol and the residue dried and weighed. I evaporated the alcoholic solution after addition of a little calcium carbonate with the object of neutralising the acids. The residue was dissolved in water, additioned with a little solution of neutral lead acetate and made up with water to 100 cM^3 in order to get the sugars in the solution in the same concentration as that in which they originally were present in the pulp. I determined polarisation and reducing sugar in this solution both before and after inversion and calculated from the figures obtained the amount of sucrose, glucose and fructose after having stated that no other sugars were present in the liquid.

I pulverised the dried residue left behind after the alcoholic extraction of the pulp and extracted part of it with cold water. This extract was evaporated to a small volume and precipitated with alcohol. The precipitate was collected on a weighed, ashless filter washed with alcohol, dried, weighed, incinerated and the loss of weight occasioned by the combustion of organic matter was recorded as dextrin after I had convinced myself by the red coloration which iodine solution produced in the solution of such a precipitate that it really was dextrin.

A second portion of the residue was hydrolysed with hydrochloric acid under pressure and the amount of glucose thus obtained calculated as starch. Finally, I determined the percentage of nitrogen and calculated from this figure the amount of albuminoids by multiplication with the factor 6,25. The figures for the different analyses follow here:

Date of the analysis	17th April	19th April	20th April	22d April	23d April	24th April
Degree of maturity	*Unripe* the skin adheres *to the* fruit	Unripe the skin loosens	Begins to ripen	Alm. ripe	Ripe	Over-ripe
% Skin	45	44	43	39	37.8	36.2
% Fleshy matter	55	56	57	61	62.2	63.8
Composition of the pulp						
Moisture	58.24	59.21	59.48	59.86	60.88	61.12
Dry substance	41.76	40.79	40.52	40.14	39.02	38.88
Insoluble in alcohol	39.41	34.06	29.58	20.98	15.30	13.00
Soluble in alcohol	2.35	6.73	10.94	19.16	23.72	25.88
Sucrose	0.86	4.43	6.53	10.50	13.68	10.36
Glucose	0.25 (glucose and fructose)	0.96	1.80	3.18	4.72	6.1
Fructose		0.90	1.53	2.70	3.61	4.8
Dextrin	trace	0.52	0.59	0.69	0.65	0.65
Starch	30.98	24.98	20.52	13.87	9.59	7.68
Albuminoïds	2.65	2.60	2.60	2.58	2.58	2.55
Ash	0.94	0.96	0.97	0.95	1.00	1.01

The skin contains much rubber, fibre and also a small amount of soluble carbohydrate and its composition calculated in 100 parts of dry substance does not vary considerably in the green and in the ripe state. The water content, however, diminished greatly during ripening so that the shrinkage of the skin is chiefly due to loss of water.

The analysis of the pulp shows large differences during the after-ripening because of the starch being rather suddenly transformed on a large scale into sucrose.

That the sugar present in the ripe fruit was really sucrose was proved by evaporating to a small volume the clarified alcoholic extract from fully ripe bananas and allowing it to crystallise. After some time it deposited crystals which were recognised to be sucrose by numerous chemical and physical tests. In the ripe fruits this sucrose becomes partly inverted or consumed by the aspiration either as such or as products of its inversion. The latter possibility is the more probable one, as, first of all, much carbonic acid is formed during the after-ripening and secondly because the fructose is in every case present in a smaller proportion than the glucose. It is evident, therefore that these two constituents are not consumed together as sucrose, but separately after the splitting up of that body and then the fructose more readily so than the glucose.

During the saccharification process a little dextrin is formed too.

b. Mango (Mangifera).

The mango fruit, as a rule, is picked when still unripe; in this state the fruits are internally white, hard, acid and flavourless, but within a few days they undergo an after-ripening process which renders them tender, full-flavoured, and yellow or orange-coloured.

This period is, as in the former case, soon followed by over-ripeness and decay.

A few mango fruits, of a variety which bears very sweet and well-flavoured fruits when ripe, were picked green, placed on a cool spot at a temperature of 28° C. and weighed every day with this result

N°.	29th Sept.	1st October	2d October	4th October
I	247 Gr.	243 Gr.	241 Gr.	240 Gr.
II	229	226	224	223
III	227	223	222	219.5
IV	249	247	246	244

Five green mangos were weighed and placed under a glass bell jar, through which a current of air free from carbonic acid was conducted which afterwards was made to pass through a Liebig potash bulb.

This latter was weighed daily and the 5 fruits only after the end of the experiment.

The 5 fruits weighed originally	1139.3 grammes
After 3 days	1121.3 „
and therefore lost in 3 days	18.0 grammes

The potash bulb increased during

the first day by	1.712 grammes
the second day by	1.276 „
the third day by	1.570 „
Or in three days	4.558 grammes

The fruits gave off 4.558 grammes or 0.40°/₀ of carbonic acid in three days.

Just as in the case of bananas a mango fruit from a parcel having practically the same initial maturity was daily analysed; and this time the analysis extended with a determination of free and total citric acid. I had previously stated that the acid in the mango really was citric acid and that no other organic acid could be found in it.

I determined the free citric acid by titration with 1/10 normal potash in the boiled fruit whilst the amount of total citric acid was determined by extracting the boiled fruit with alcohol and precipitating the citric acid in the alcoholic liquid by means of barium acetate. The precipitate was filtered off, washed, incinerated and finally, I determined the carbonic acid in the ash which was of course equivalent to the total citrate in the precipitate. The figures obtained follow here.

The yellow colouring matter, which is produced during the ripening process, shows the same reactions as the carotine from carrots, the same spectroscopic appearance and in fact resembles it in every respect.

During the after-ripening the starch is transformed into sucrose, which later on becomes hydrolysed and splits up into glucose and fructose. In the beginning of the process the fruit liberates water but this constituent increases afterwards owing to the combustion of the carbohydrates. The citric acid is vigorously attacked and the decrease in the acid taste during the after-ripening is not due to an increase

Date of the analysis	29th Sept.	1st October	2d October	4th October
Degree of maturity	Unripe	Almost ripe	Ripe	Over ripe
Moisture	83.34	82 95	81.95	83 20
Dry substance	16.66	17.05	18.05	16 80
Soluble in alcohol	6 36	15.18	15.54	14.70
Insoluble in alcohol	10 30	1.87	2 51	2.10
Sucrose	2.57	10.50	12.27	9 31
Glucose	0 60	1.53	1.30	2.10
Fructose	1.90	2.10	2 01	2 60
Starch	8 53	0.55	0	0
Free citric acid	1 36	0.34	0 25	0.10
Total citric acid	1.31	0.37	0 21	0 10
Ash	0.42	0.44	0.41	0.43
Albuminoids	0 80	0 80	0 75	0.73

in the sugar content, nor to a neutralisation of the acid but solely to combustion and thus destruction of the organic acid itself.

C. Tamarind (Tamarindus).

The tamarind fruits remain on the tree untill they are fully ripe and thus do not undergo any after-ripening process after being plucked or shaken off. In the unripe state the flesh is white and hard and fills the whole pod so that the woody skin is firmly attached to it. Later on, when the fruit ripens, the flesh becomes tender and brown and owing to evaporation, shrinks in such a way that a large empty space exists between the dry pulp and the hard skin. The composition of the pulp of tamarind fruits in several stages of ripeness is given here. (see p. 80).

In this case too the starch has become transformed into sugar, during the ripening but this time not into sucrose, but into a mixture of glucose and fructose. At the same time a great deal of water was evaporated, causing the fruit to shrink in its envelope and finally much acid was consumed by respiration, since the amount of total tartaric acid in the dessicated fruit was smaller than that in the so much more juicy one of a month before. The increase of the percentage sugar after the period of maturity is due to the

Date of the analysis	11th May	27th May	20th June	15th July
Degree of maturity	Green	Almost ripe	Ripe	Dessicated
Dry substance	15.86	38 46	40 92	76.20
Moisture	84.14	61.54	59.08	33.80
Glucose	0 40	10.10	20.4	25.10
Fructose	0.33	5.10	11 6	10.6
Fibre and pectin	3 27	8.10	7.90	14 57
Starch	3 33	1.25	0	0
Free tartaric acid	3 25	15.8	14.6	—
Total tartaric acid	4 85	18.1	16 4	14.4
Potassium bitartrate.	4.00	5.76	4.50	—

strong concentration by evaporation, because no fresh formation of sugar can possibly have taken place in so dry a fruit.

d. Sapodilla (Achras sapota)

The fruits are plucked tree-ripe; in which state they are green and hard, and contain tannin and gutta-percha dissolved in the sap, which render the fruit unfit for eating. After they have been preserved in bran, the gutta-percha as well as the tannin, become insoluble and the fruit itself gets tender, full-flavoured and palatable. On examining sections of the fruit one sees the coagulated gutta-percha as a series of white strings, while the tannin is deposited as insoluble matter in some cells.

The analyses of such fruit in a tree-ripe and full-ripe condition are given here.

	Tree-ripe	Full ripe
Moisture	74.76	75.20
Dry substance	25.24	24.80
Sucrose	7.80	7.02
Glucose	2.85	3.7
Fructose	2.70	3.4
Starch	Absent	Absent
Pectine	3.34	4.00
Albuminoids	0.45	0.40
Ash	1.50	1.50

Unlike the after-ripening of the former three fruits, this one is not due to saccharification of starch. The amount of sugar before and after the full ripening is the same, but in this case the fruit has become palatable by the softening of the hard pectin and by the deposit of tannin and gutta-percha from the juice as insoluble bodies.

I have to mention here that I did not find lactose in this fruit which has been stated by BOUCHARDAT as being one of its constituents. They, however, contain much pectin and owing to the presence of this body the juice yielded a fair amount of mucic acid on oxidation with nitric acid; this renders the supposition probable that this acid, considered by BOUCHARDAT as an evidence of the presence of lactose, has simply come from the pectin.

II. Agents of the saccharification during after-ripening.

When studying the fruits which come first into account in the research under consideration, viz. the banana and the mango fruit, we found in a certain stage of the development a rather sudden transformation of starch into sucrose, followed in a later stage by inversion and partial transpiration of the products of inversion. From experiments on the determination of the carbonic acid in the atmosphere in which this sudden transformation took place, I came to the conclusion that just the period of the rapid saccharification coïncided with a strong development of carbonic acid, or with a powerful oxidation and degradation. At the same time the moisture on the inside of the glass bell jar in which the fruits ripened showed that a copious evaporation had accompanied the oxidation.

The figures for the carbonic acid from the bananas showed on the second day a strong development which decreased very soon, whilst those for the mangos remained somewhat stationary for the three days under observation. These data correspond very well with the more rapid after-ripening of the former fruits during this experiment in which they turned from green into yellow even on the second day.

The transformation is therefore accompanied by oxidation and I tried to check it by excluding the fruits from the free access of oxygen. To this end I covered a few green mango and banana fruits with collodion and kept them together with a few similar fruits not covered with an impermeable layer. The fruits covered with collodion did not ripen well, and were converted into decayed masses, while locally the wrinkles occasioned by the dying off of the fruit caused the collodion layer to burst and thus made the experiment unreliable. Moreover it might well be that the decay was not only to be ascribed to the exclusion of oxygen but to the hindered evaporation which would be injurious to the fruit.

In order to elucidate this point, a few bananas were placed in a tube, through which a current of nitrogen passed, while at the same time some other bananas from the same part of the bunch were kept in the ordinary atmosphere. When the latter had become yellow, tender and ripe, those in the nitrogen tube had still retained their green appearance. The analyses of the peeled fruits of the two parcels yielded these figures.

	In nitrogen	In air.
Moisture	70.54	68.36
Insoluble in alcohol.	25.90	11.06
Soluble in alcohol.	3.57	20.58
Sucrose	0.31	13.66
Reducing sugars	0.94	4.80

It followed then, that the after-ripening in the air had gone on uninterruptedly whilst the fruits kept in the nitrogen atmosphere had remained unchanged and had preserved their starch content; so that free access of oxygen is an indispensable condition for the saccharification of starch in the fruit.

The following experiments were undertaken with a view to ascertaining whether this saccharification was brought about by a vital process or by the action of some diastatic ferment present in the fruit.

A jelly consisting of isinglas and agar agar of such a composition that it was solid at the ordinary temperature was mixed with 1°/₀ of starch, poured into a series of Petri dishes and sterilised. Slices of green mango and banana fruit or pieces of half ripe tamarind fruit were placed on the stiff jelly in some dishes and on that of others figures and letters were traced with a pencil dipped in mango-juice. After two, or sometimes more, days the particles of fruit were removed and the jelly covered with a very dilute solution of iodine in potassium iodide which after having remained there for a minute was washed off. In every case not only the spot where the fruit had been placed or where the pencil strokes had been applied, remained white, but all round a white stain spread out, lined with a red border which gradually faded into the surrounding blue coloration of the still unattacked starch. The longer the dishes had been allowed to stand, the larger was the white stain. In every one of these cases, therefore, a diastatic ferment had diffused from the fruits and from the juice, which had transformed all the starch, it could get hold of through the state of dextrin into sugar.

When the iodine solution was allowed to act too long on the jelly, the iodine penetrated through the surface layer and reached the lower one, where the starch was still unattacked, thus colouring the whole dish blue. Finally pieces of banana and tamarind fruit were placed on slices of sterilised potato; the result was that the saccharification of the starch caused more or less deep cavities to appear in the places where the fruits had been applied.

All this however is not yet a direct proof that the saccharification has been occasioned by a ferment; and in order to make this clear I immersed slices of banana into alcohol, left them there during a couple of days, then took them out, expelled the alcohol by means of a current of sterilised air and placed them again in Petri dishes on a layer of starch emulsion stiffened with isinglass and agar agar. Though not so rapid as in the case of the much more juicy fresh fruit, yet also here the ferment diffused through and after the application of the iodine solution the white stains with the red borders became visible.

A quantity of mango juice was added to a boiled and re-cooled solution of 3 % starch at 50° C. and kept at that temperature for some time. The liquid, which, at the outset, had given a deep blue reaction with iodine solution only became red when at the end of the experiment this test was repeated; this coloration did not undergo any change even if the mixture was kept for some time longer or if a fresh quantity of mango juice was added. The total amount of sugar, contained in the liquid (for the mango juice itself had also contained sugar) was higher after the reaction than before, which showed that the mango juice had contained a diastatic body with power to transform starch into dextrin and into sugar.

Now the question still remained which sugar is formed in the laboratory outside of the living organism.

The ripening fruits and their juices already contain so much sugar, which mixes with the small amount of sugar formed by the saccharification of the starch that the proper identification of that latter portion is extremely difficult if not impossible.

In order to eliminate the influence of the already existing sugar, ripening banana fruits were peeled and repeatedly triturated with alcohol and the extracted pulp, which contained as little sugar as possible was pressed and brought into glycerin. After a few days the amount of sugar and its nature was ascertained in the glycerin by

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polarimetric and copper tests both before and after inversion. Next 100 grammes of this glycerin were mixed with a 3 °/₀ starch solution, warmed to 40° C. and kept at that temperature for a couple of hours. After that the dissolved starch and dextrin was precipitated with alcohol, filtered, a pinch of calcium carbonate was added to the filtrate to prevent inversion by the slightly acid reaction of the filtrate, and the alcohol was evaporated off. The syrupy residue was dissolved in water, diluted to the volume of 100 cM.³ and used for the determination of the sugars by the polarimeter and Fehlings solution before and after inversion.

The original glycerin solution had contained 0.17°/₀ of glucose both before and after inversion, while after the treatment with starch 100 grammes of the solution contained 0.60 grammes of reducing sugars before inversion and 0.67 after that operation, which shows that 0.43 grammes of glucose and 0.07 grammes of sucrose (?) have been formed from the starch by the ferment. The polarisation of the solution was + 0.9 before and + 0.4 after inversion, giving evidence, that notwithstanding the precipitation with alcohol, a small amount of starch or dextrin has still remained dissolved.

At any rate from the fact that the exclusion of oxygen prevents the saccharification of the starch in the fruit and from the negative results of the experiments on formation of sucrose by means of fresh juice and of the precipitated and re-dissolved ferments, it follows that the rapid transformation of starch into sucrose during the after-ripening of some fruits is a vital process and not a consequence of the action of some ferment contained in the fruit which, just as diastase forms maltose from starch, could be isolated to form large quantities of sucrose from any kind of starch in the laboratory.

Mathematics. — *"Congruences of twisted curves in connection with a cubic transformation."* By Prof. JAN DE VRIES.

(Communicated in the meeting of May 30, 1908).

§ 1. If x_1, x_2, x_3, x_4 are the coordinates of a point X with respect to a tetrahedron having O_1, O_2, O_3, O_4 as vertices, then

$$x_1 x'_1 = x_2 x'_2 = x_3 x'_3 = x_4 x'_4$$

determines a cubic transformation which transforms the right line

$$x_k = \lambda a_k + \mu b_k$$

into a twisted curve ω^3, represented by

$$\varrho x'_k = \frac{1}{\lambda a_k + \mu b_k}.$$

The congruence Γ of the curves ω^3 through the five points