THE FRUIT OF BASSIA LONGIFOLIA.

The Changes taking place in its Composition after it is gathered.

By Gilbert J. Fowler and Talwar Dinanath.

INTRODUCTION.

During an investigation into the biogenesis of oil in the seeds of *Bassia longifolia* (Mawha, Mahua, Mowra, Illipe or Ippe as it is called in different vernaculars) it was observed incidentally that, near the ripening stage, the fruits developed saccharine matter which increased rapidly after gathering. This observation we believe to be new, and therefore we deemed it advisable to investigate systematically the changes involved.

Preliminary experiments showed that the proportion of sugars was considerable, and in view of the fact that large quantities of alcohol are derived from the flowers of the mahua tree [1] it was thought that if the fruits could be proved to yield an additional supply of alcohol important industrial possibilities might follow.

It would obviously add considerably to the potential value of the mahua tree, and encourage systematic planting if it could be shown that, in addition to the sugar contained in the ripe corollas of the flower and the oil recovered from the seeds, the husks, hitherto wasted, were also a source of alcohol.

The tree begins to bear fruit at about the end of May or beginning of June. On the 21st of June when our original researches began the fruits had already developed to a stage when the seeds contained some oil. In the beginning of August the fruits are sufficiently ripe to fall in a slight wind. It was observed at this stage that the fruit was attacked at night. Local information described the enemy as Chikurayee (Telugu), i.e. the flying fox. In the morning, the seeds rejected by this animal are collected from the ground. In the batch of trees which we had under observation, the fruits after this stage began rapidly to vanish, and, on the 23rd August, we had difficulty in obtaining even thirty fruits from one tree.

[1] G. J. Fowler and others. *This Journal*, 1920, 3, 81.

The fruits at this stage are hard and full of latex, but when allowed to remain overnight after plucking, the latex disappears and the fruits become softer, till on the third day after plucking they are so pulpy that the slightest pressure will bring out the juice. The seeds, which are very difficult to remove from the fresh fruit, are now easily liberated from the surrounding husk on pressing the fruit. On the fifth day after plucking, the fruits become slightly discoloured and show signs of rotting.

EXPERIMENTAL.

GENERAL METHODS.

No standard methods of analysing plant substances having been devised, we propose to give in detail the procedure and methods of analysis which we followed, together with our reasons for adopting them.

A large number of fruits along with the stalks were brought from a single tree near Yeswantpur Railway Station (about half a mile from the Institute) which had escaped depredation, and left undisturbed at an average temperature of 25° C. on the 11th September, 1922. Within two hours of plucking, about a dozen fruits were removed from the stalks and weighed. The seeds were taken out carefully and washed three times with alcohol, the washings being added to the alcohol in the extraction flask. The husks were disintegrated with a clean sharp knife and transferred to a 250 c.c. extraction flask, containing 125 c.c. of 90 per cent. alcohol and 1·25 c.c. of strong ammonia (Sp. Gr. 0·880). The flask was then heated under a reflux condenser for three hours and left overnight. The next morning extraction was continued for five hours, the extract after cooling being pressed by hand through a muslin cloth in a large porcelain dish. The residue was washed several times with distilled water and the washings pressed out as before ; as the filtrate contained some fine whitish particles it was filtered again on a Buchner funnel, to which the residue on the muslin was then transferred and washed again twice or thrice with water. With this filtrate, generally faintly alkaline (if not, sufficient ammonia was added), a few drops of toluene were shaken and it was analysed for sugars and tannins, whilst the residue was examined for starch, as given under the heading ' Detailed Methods of Analysis '.

In this method of alcohol extraction, we have followed Davis, Daish and Sawyer [1] who have shown that the destruction of enzymes by this method is instantaneous ; the ammonia not only neutralises

[1] *J. Agri. Sci.*, 1915-16, 7, 263.

the acids in the fruit, thus obviating inversion of the sugars, but owing to rapid diffusion into the plant cells it ensures complete destruction of the enzymes. Indeed our results seem to bear out their observation, since it would have been impossible to get such concordant results if the destruction of the enzymes were not instantaneous and complete, or if other hydrolytic changes had proceeded during the operation. The destruction of enzymes and bacteria is very important, since they alone are responsible for the changes investigated in this paper.

This method of alcohol extraction was followed throughout in all its details on the succeeding four days, except that after the 12th September, the knife was not used to chop the fruit, since the fruit had become very pulpy, and it was found that a gentle tearing with the fingers involved less loss of juice.

For the estimation of the dry matter on the successive days, three or four fruits were taken from the same heap and at the same time as the dozen or so taken for alcohol extraction, weighed and put at once into a hot water oven at 90–95° C. They were dried there for about a week, the shell broken and the seeds and husk weighed separately.

Three or four fruits were also taken, the seeds removed and the husks ground with fine washed pure sand for about half an hour. 50 c.c. distilled water were then added. A crystal of thymol was dropped in, crushed with a pestle, and the whole left for about two hours. It was then filtered through a thin muslin cloth and the filtrate analysed for enzymes as given under the heading ' Detailed Methods of Analysis '.

The filtrate was turbid. No attempt was made, however, to clarify it, several writers having shown that the enzymic activity, decreases rapidly with repeated filtrations (Cf. [1, 2, 3].) Only thymol, moreover, was used as an antiseptic, since maltase with which we were, directly concerned is very sensitive to other antiseptics. [4, 5.]

A large number of fruits were collected on the 29th August from the same tree and on the 30th about twelve fruits without stalks were weighed at about 2 p. m., deprived of their seeds and crushed with distilled water in a wide glass basin. The pulp was then transferred to a clean washed muslin cloth, and pressed out several times after being wetted with warm distilled water. This was done at least four or five times. The seeds were washed thoroughly with

[1] Robertson, Irvine and Dobson, *Biochem. J.*, 1909, **4**, 258.
[2] Davis, *Biochem. J.*, 1916, **10**, 31-48.
[3] Daish, *Ibid.*, pp. 49–55
[4] Onslow, *Practical Plant Bio-Chemistry* (1920), p. 76.
[5] Croft Hill, *J. Chem. Soc.*, 1898, **73**, 634.

warm distilled water and the washings added to the fermentable liquid. The pressed liquid was whitish in colour (except No. 5 which was brownish) and had a quantity of greenish fine matter in suspension which generally settled after keeping for some days. The extract was made up to a definite volume, the specific gravity was determined with a hydrometer, and the liquid sterilised at once in the steam steriliser for one to two hours. Such extracts were taken on five successive days from the same heap of fruits ; all the sterilised extracts being kept in the ' Media room ' till required for inoculation. They were then all inoculated on the same day and the alcohol estimated as given under the heading ' Detailed Methods of Analysis '.

The total dry matter was calculated, as in the previous case, by drying a weighed quantity of fruits daily.

Detailed Methods of Analysis and Results.

Sugars.—The alcohol extracts obtained as described above amounted with washings generally to 1·5 or 2 litres. It was necessary, therefore, to reduce the volume considerably before attempting to analyse the sugars. Davis has described an apparatus[1] which he devised for his work and which he recommends generally for concentrating alcohol extracts of plant substances. We found, however, that our extracts did not froth so much as those described by him, and, by taking the following precautions, we were able to concentrate our extracts to the required extent, by distilling under a pressure of 60 mm. at 50-60° C. over a water-bath in an ordinary vacuum distilling apparatus.

A 1000 c.c. flask with a long neck was used, so that the slight bumping, which still occurred in spite of the presence of glass beads, did not carry the liquid into the receiver. The amount of liquid never exceeded 200-300 c.c. A dropping funnel admitted the extract slowly as the distillation proceeded.

Four or five hours were required to reduce the volume of the extract to about 150 c.c. The extract was then washed out of the flask, the dropping funnel, delivery tube, etc. washed thoroughly and the washings added to the extract and the whole made up to 200 c.c. in a measuring flask. The extract was generally faintly alkaline, but if not, a little ammonia was added, followed by a few c.c. of toluene.

Before beginning the regular analysis of sugars a qualitative examination of the clarified extract was made to ascertain what sugars

[1] *J. Agric. Sci.*, 1912-13, 5, 434.

were present. The osazones were prepared from 5 c.c. of the clarified extract according to Fischer's method [1] by heating it in a test-tube with 0·5 gram phenylhydrazine hydrochloride and 0·75 gram sodium acetate in a boiling water-bath for one hour. The insoluble osazone was identified as that of glucose, whilst the product deposited by the cooling filtrate was recognised as maltosazone. Preliminary experiments had shown that sucrose also existed in the mixture, besides the reducing sugars.

No really satisfactory method exists for the estimation of sugars in plant extracts containing maltose. The best seems to be the one recommended by Davis and Daish [2] in which special maltase-free yeasts are used to ferment away the rest of the sugars after which the maltose can be estimated by the customary method; but the method is tedious. We used that recommended by Haas and Hill [3] in which citric acid and hydrochloric acid are used separately to hydrolyse two portions of the original mixture. In the first only cane-sugar is hydrolysed and maltose left untouched, [4] in the second case maltose and cane-sugar are both hydrolysed. The necessary calculations are as follows:—

If a = total reducing power before any hydrolysis,

b = reducing power after hydrolysis with citric acid, and

c = reducing power after hydrolysis of the original mixture with HCl,

then $(b-a) \times 0{\cdot}95$ = cane-sugar,

$(c-b) \times 2{\cdot}32$ = maltose, and

a − (maltose × 0·62) = reducing sugars other than maltose.

In a duplicate series of experiments with pure sugars to test the accuracy of the method, it was found that maltose and reducing sugars (other than maltose) determined by this method appeared slightly low; but since in our investigation we were concerned less with the absolute quantities of the different sugars than with the proportion in which they were present from day to day, we decided to adopt this method. Care was taken to conduct the analysis on all the five days under exactly the same conditions.

[1] Browne's *Hand Book of Sugar Analysis*, p. 344.

[2] *J. Agric. Sci.*, 1912-13, **5**, 437-68.

[3] Haas and Hill, *Chemistry of Plant Products*, vol. i, 1921, p. 98.

[4] The use of citric acid alone for the hydrolysis of cane-sugar in a mixture containing maltose has also been strongly advocated by Davis and Daish as the only safe method (*Loc. cit.*, p. 447). Kluyver took objection to this and maintained with Jelowitz (*Zeitsch. angew. Chem.*, 1895, 208) that hydrochloric acid did not affect maltose. Davis, however, in a subsequent paper (*J. Agric. Sci.*, 1914, **6**, 413) in reply to Kluyver, reaffirmed his conclusion and proved his point with the help of a new series of experiments.

50 c.c. of the extract were transferred to a small conical beaker and 1·2 c.c. of basic lead acetate (prepared according to the A.O.A.C. method [1]) added.[2] It usually coagulated at once, otherwise the whole beaker was placed for a few minutes in a warm water-bath, when the coagulation took place rapidly. The liquid was filtered and the precipitate washed several times with small quantities of warm distilled water. The filtrate was freed from lead with solid sodium phosphate as recommended by Englis and Tsang [3] till no milkiness appeared on the addition of another crystal of sodium phosphate. The liquid was then filtered, the precipitate washed several times with small quantities of distilled water and the filtrate made up to 100 c.c. in a measuring flask. This was called A.

The reducing power of A was estimated volumetrically by the Ling-Rendle-Jones method [4, 5] using ferrous thiocyanate as indicator. This method was preferred to the gravimetric methods, since opinion is divided as to the best way of weighing the reduced copper oxide.

It is true that the end-point in the volumetric method is rather difficult exactly to define, but we have been able, with experience, to determine it within such limits that two successive readings rarely differed by more than 0·5 c.c. We found, moreover, that in the case of the solutions under examination, the disappearance of the blue colour gave as good an indication of the end-point as the ferrous thiocyanate itself.

25 c.c. of A was then treated with 10 per cent. citric acid crystals and heated in a boiling water bath for fifteen minutes. It was then cooled immediately, the liquid nearly neutralised with pure solid Na_2CO_3 and made up to 50 c.c. This was called B. The reducing power was determined as with A, the reducing sugars being calculated as glucose.

25 c.c. more of A were taken, 10 per cent. HCl added and the flask heated in a boiling water-bath till the temperature of the liquid inside rose to 70° C. It was then kept at 70° C. for fully ten minutes, cooled

[1] Allen's *Commercial Organic Analysis* (4th edition), vol. i, p. 308.

[2] It is generally believed that basic lead acetate carries down with it considerable quantities of lævulose. Davis (*J. Agric. Sci.*, 1916-17, 8, 7-15) has shown, however, that if an excess of the basic lead acetate is avoided, and the liquid is not left for any considerable time in contact with the lead solution, this precipitation of lævulose does not occur.

[3] *J. Amer. Chem. Soc.*, 1922, **44**, 865-7.

[4] *Analyst*, 1905, **30**, 182.

[5] *Ibid.*, 1908, **33**, 160.

[For 4 and 5 cf. also *Technical Methods of Chemical Analysis*, Lunge and Keane, 1914, vol. iii, Part 2, p. 564.]

immediately, neutralised with pure sodium carbonate and made up to 50 c.c. This was C. The reducing power of this also was determined as with A and B. The results are given in Table I.

Tannins.—The tannins were determined day by day in the alcoholic extract obtained as above, along with the sugars. Lowenthal's volumetric method as modified by Procter [1] was used at first (the detanning was done with 2 per cent. gelatine solution containing sodium chloride and sulphuric acid as recommended by Hunt), but the results were not concordant. We repeated the estimation therefore with the gravimetric method adopted by the A.O.A.C., [2] pp. 43-4. The total solids in the alcoholic extract were first determined by evaporating 20 c.c. of the extract and drying at 95° C. 50 c.c. of the extract (made up to 200 c.c.) was then detanned by shaking for ten minutes in a shaker with 40 grams of wet chromed hide powder, squeezed through muslin, shaken with kaolin and filtered till clear. The total solids were again determined in an aliquot portion of the filtrate, the difference between the total solids before and after detanning giving the amount of tannins in the extract. The results are given in Table I.

Starch.—The residue on the Buchner funnel obtained as mentioned above was dried thoroughly in a hot-water oven, ground as finely as possible and kept in a desiccator. The starch in this was estimated according to the A.O.A.C. method [2] (p. 95) in which the dextrins and other water-soluble matter is first removed by thoroughly washing with distilled water [3] and the starch then hydrolysed with hydrochloric acid. The dextrose so formed was estimated by the Ling-Rendle-Jones method as described above, the result multiplied by 0·90 giving starch.

Davis and Daish[4] have criticised this method and advocate, instead, the use of Taka-diastase for hydrolysing starch. Horton, [5] however, has subsequently published a long paper proving that the Taka-diastase method as recommended by them is not implicitly reliable. In the face of this controversy we thought it best to follow the old-established acid hydrolysis method especially when, as noted elsewhere, we sought only comparative results.

The results are given in Table I. To calculate the percentage of starch on the dry husk, 20 c.c. of the alcohol extract were

[1] Procter, *Leather Industries Laboratory Book*, second edition, 1908, p. 228.
[2] Association of Official Agricultural Chemists (A.O.A.C.), *Official and Tentative Methods of Analysis*, 1921.
[3] Cf. also C. O'Sullivan, *J. Chem. Soc.*, 1884, **45**, 1
[4] *J. Agric. Sci.*, 1914, **6**, 152
[5] *J. Ibid.*, 1921, **11**, 240.

evaporated on a water-bath and then dried thoroughly in the hot-water oven at 95° C. in a clean dry flask. The flask was weighed before and afterwards. From this the total alcohol-soluble matter was calculated, and this subtracted from the total weight of dry husk gave the amount of matter insoluble in alcohol, about three grams of which was used for the estimation of starch.

Enzymes.—Amylase, invertase and maltase alone were looked for in the extract (obtained as described above) on successive days. The method of procedure adopted was on the lines of Kastle and Clarke [1] and consisted of preparing in five separate 50 c.c. flasks the following systems :—

M. 0·5 gram maltose + 30 c.c. distilled water + 10 c.c. extract (for maltase).

C. 0·5 gram cane-sugar + 30 c.c. distilled water + 10 c.c. extract (for invertase).

S. 30 c.c. 1 per cent. starch solution + 10 c.c. extract (for amylase).

A. 30 c.c. distilled water + 10 c.c. extract (active control).

m. 0·5 gram maltose + 40 c.c. distilled water (maltase control).

The flasks were plugged with cotton wool and kept in an incubator at 37° C. for twenty-four hours, after which they were kept for another twenty-four hours at room temperature (average 25° C.) 10 c.c. from each was then transferred to separate flasks, 2 c.c. mixed Fehling solution added to each and all heated at once on a piece of asbestos board. A comparison of the amount of copper reduced in each case, with the active control (A) and with maltase control (m), indicated approximately the amounts of the enzymes present. The object has been mainly to demonstrate the absence or presence of the ferment. The results are given in Table II.

Fermentation to alcohol and its estimation.—The five sterilised extracts obtained as described above were inoculated with a pure culture of *S. Thermantitonum* [2] specially prepared in an actively fermenting condition by Mr. B. N. Bannerji. The flasks were then kept in the incubator at 37° C. Within an hour, gas bubbles began to appear. The flasks were left in the incubator for five or six days

[1] *Amer. Chem. J.*, 1903, **30**, 421-7.

[2] Sykes and Ling, *The Principles and Practice of Brewing*, pp. 391-3.

till all fermentation (as shown by the evolution of gas) stopped, after which the alcohol formed was distilled off and alcohol estimated as usual by taking the specific gravity with a specific gravity bottle. The results are given in Table V.

Discussion of Results.

An examination of the Tables I and II shows that cane-sugar increases to a maximum on the third day after plucking, after which it falls off. The maltose is absent on the first day (i.e. just after plucking), but is present on all the succeeding days. Its proportion, however, is never large and it increases or decreases irregularly until the fifth day, when it increases, suddenly. The reducing sugars (other than maltose) Table I, 6c, on the other hand, rise steadily to the end. The total alcoholic extract rises till the fifth day when it falls. The starch falls rapidly in the first two days after which, although it still decreases, the loss is not material.

Table II shows that the enzyme maltase is very active on the first three days; on the fourth it grows less and on the fifth its presence is doubtful. The enzyme amylase is very active on the first day, but, after nearly twenty-four hours, its activity decreases and remains almost constant till the last. The invertase similarly is most active on the first two days, after which it gradually falls, and is almost absent on the last day.

These two tables, which are in a way a check one upon another, seem to indicate that amylase and maltase break up starch into dextrins and dextrose, the invertase acting as a synthetic enzyme and converting a major portion of the products into cane-sugar.[1] These reactions are very powerful on the first two days, after which, the amylase having fallen, the starch degradation is retarded and invertase begins to hydrolyse cane-sugar. The fact that the proportion of maltose is never large, and that its quantity rises and falls in no definite way, coupled with its absence on the first day, shows that it is only an intermediate product in the degradation of starch going on in the fruit. The enzyme maltase transforms it as soon as it is formed from starch and consequently when this enzyme is almost absent on the last day, the quantity of maltose at once shows an increase.

These results are borne out by the observations of Tallarico[2] who found during an investigation on the hydrolytic and catalytic ferments acting during the process of ripening of fruits, that invertase

[1] Fowler, *Bacteriological and Enzyme Chemistry*, p. 270.
[2] *Chem. Zentr.*, 1908, 1563-4.

is absent when green, but is intense during ripening and then gradually disappears and that the amylase is most active in the green stage and at the beginning of the stage of ripening. Geerligs similarly [1] in an investigation on the changes in the composition of bananas, mango and tamarind found an increase in the sugar content on ripening due to the conversion of starch into sugar. Kayser,[2] also, as a result of his studies on the occurrence and products of alteration of cane-sugar in plants, reached the conclusion that this carbohydrate results from starch by the direct action of a diastatic ferment and that it is then transformed into invert sugar by the action of a ferment similar to invertase before it is consumed in the respiratory processes in the plant.

Martinand [3] has shown that grapes contain sufficient invertase to invert the whole of the sucrose present. Later on[4] he examined the different organs of the vine, cherries, currants and pomegranates and reached the same conclusion with regard to the presence of invertase. More recently McHargue[5] has studied the chemical changes occurring during the ripening of the wild-goose plum and has concluded from his results that cane-sugar plays a very important part in the ripening, and suggests that the fruit is just ripe when it contains the maximum amount of cane-sugar. The enzyme invertase, he found to be most active in passing from the ripe to the over-ripe stage. The universal presence of invertase and the important part it plays in the plant metabolism have also been emphasised by Kastle and Clark (*loc. cit.*) who give a number of references to original papers dealing with invertase in plant life.

Table I shows that tannins are almost constant till the third day, when the general decomposition sets in, as shown above, and then they fall. Otto and Kooper[6] observed a similar decrease with *Pinus spinosa* etc., and they attribute the change to the tannins being converted to some oxidised compounds.

Table III, which is another check upon Tables I and II, shows that the amount of fermentable sugars increases to a maximum on the third day, after which it gradually falls. This would seem at first sight to contradict the results in Table I, if the figures under 6*c* were taken to mean fermentable sugars. It is possible, however, that after the

[1] *Proc. K. Akad. Wetench*, Amsterdam, 1908, 11, 74-84. (Cf. also *J. Chem. Soc.*, 1908 A, ii, 977.)

[2] *Landw. Versuchs, Stat.* 1883, **29**, 461-73.

[3] *Compt. rend.*, 1900, **131**, 808-10.

[4] *Ibid.*, 1907, **144**, 1376-8.

[5] *J. Amer. Chem. Soc.*, 1916, **38**, 718-22.

[6] *Z. Unters. Nahr. Genussen*, 1910, **19**, 10-13. Cf. also *J. Chem. Soc.*, 1910 A, ii, 233.

third day when the general decomposition sets in, the figures obtained under this column may also include the Fehling-reducing end-products of decomposition, which will not ferment, but which reduce Fehling solution, and thus come in the same category as glucose and other monosaccharides with respect to copper reduction.

INDUSTRIAL POSSIBILITIES.

It is evident from Table III that the sugars formed in the course of ripening of the fruit under the conditions investigated in this paper are fermentable and that the yield is a maximum on the third day after plucking, when the quantity of absolute alcohol obtained amounts to 10·2 per cent. calculated on the weight of dry husk. This corresponds to about 5 gallons of absolute alcohol per ton of original fruit (without drying and with seeds) or to about 29 gallons per ton of dry husk apart from seeds. This is more than equivalent to the alcohol recoverable from dry wood waste or megasse[1] and does not, moreover, entail any expenses of preliminary hydrolysis such as are inevitable in the case of wood waste, etc.

The fermentation above described is evidently analogous to the fermentation of the pulp surrounding the cacao bean.

It is computed that seven or eight million gallons of 'sweatings' from the cacao bean run to waste every year and if this fermentation could be centralised and scientifically controlled it would constitute a valuable source of alcohol or vinegar.[2]

Considering that the husks of the mahua fruit are at present an entirely waste product, it would seem worth while to utilise them also in this way. It is suggested that the fruits might be gathered near their ripening stage and allowed to rest for two or three days, when the seeds should be extracted from the pulpy fruits by gentle crushing between rollers or in some other simple mechanical way and the pulp so obtained sterilised either with or without filtration and suitably fermented.

SUMMARY.

1. It has been shown that the fruits if plucked when near the ripening stage contain small amounts of sucrose and Fehling-reducing sugars, which increase considerably if the fruits are allowed to remain at the ordinary room temperature for a day or two.

[1] Fowler and Banerji, *This Journal*, 1921, 4, 241.

[2] A. W. Knapp, *Cocoa and Chocolate*, p. 57.

2. That the quantity of cane-sugar is at a maximum on the third day of resting after which evidently decomposition sets in and the cane-sugar and total fermentable sugars decrease.

3. That the quantity of starch decreases very rapidly in the first two days of resting.

4. That the tannins are almost constant till the third day when they begin to disappear.

5. That these changes in the composition of the fruit can be explained by assuming the degradation of the starch of the fruit by the enzymes present.

6. That amylase is very active during the first day, invertase during the first two days, and maltase during the first three days after which they all gradually diminish in effect.

7. That it is worth while to collect the fruits near their ripening stage, allow them to rest for three days and after separating the seeds from the now pulpy fruits, scientifically to utilise the pulp as a source of alcohol.

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TABLE I.

No.	—	I.	II.	III.	IV.	V.
1	Date of analysis	11-9-22	12-9-22	13-9-22	14-9-22	15-9-22
2	Weight of fruits taken in grams	156·5	118·0	121·5	116·0	107·0
	Number of fruits	8	6	6	7	8
3	Weight of fruits taken for determination of dry matter ... Grams	60	54·5	47·0	46·0	40·5
	Number of fruits	4	3	3	3	3
	Weight after drying in grams ... ... Total	19·4	18·2	14·9	15·1	14·5
	Do. seeds	10·2	10·0	7·9	7·2	7·8
	Do. husk	9·2	8·2	7·0	7·9	6·7
4	Weight of dry husk in (2) calculated from (3) grams	24·0	17·75	18·09	19·92	17·70
	Percentage	15·4	15·0	14·9	17·2	16·5
5	Total alcohol extract from (2) grams	5·84	...	8·45	10·36	8·37
	Do. percentage on dry husk	24·33	...	46·72	52·00	47·26
6	Sugars expressed as percentages on the dry husk, (*a*) cane-sugar ...	4·6	...	16·32	15·59	14·37
	Do. (*b*) maltose ...	...	...	2·39	2·22	3·8
	Do. (*c*) reducing sugars	6·13	...	8·30	8·46	10·41
7	Starch in percentage on dry husk	35·69	...	6·77	5·62	5·67
8	Tannins :—(*a*) Difference (weight in grams) between total solids in alcohol extract, before and after detanning ...	0·748	...	0·562	0·082	0·098
	(*b*) Percentages on dry husk	3·12	...	3·11	0·41	0·55

TABLE II.

Enzymes.

No.	Date	M Maltase	S Amylase	C Invertase	A Active Control	m Maltase Control
II.	12-9-1922	(14-9-1922) Clear red ppt. *Maltase present.*	Cf. A. Iodine test indicates dextrin. *Amylase present.*	Red ppt. appearing slowly. *Invertase present in traces.*	Indefinite dark ppt.	Red ppt. but not so much as in M.
III.	13-9-1922	(15-9-1922) Abundant ppt. More than in II above. *Maltase present.*	Incomplete precipitation. *Amylase present but less than in II.*	Ppt. a little more than in S or A. *Invertase present.*	Cf. S.	Ppt. but less than in M.
IV.	14-9-1922	(16-9-1922) Ppt. less in amount and less well defined. *Maltase present but less than in III.*	Definite red ppt. more than in A. *Amylase present.*	Ppt. similar to A but a little more in quantity. *Traces of invertase present.*	A red granular ppt. on the bottom. Upper liquid yellowish. (*A and m combined ppt. is less than in M.*)	Like M but ppt. much less. It is fine and adheres to the sides.
V.	15-9-1922	(17-9-1922) Slight dark ppt. No definite reduction. *Presence of maltase doubtful.*	Slight red ppt. *Traces of amylase present.*	Ppt. almost equal to A. *Presence of invertase doubtful.*	A little red ppt.	...

TABLE III.

Alcohol Fermentation.

			I	II	III	IV	V
			30-8-22.	31-8-22.	1-9-22.	2-9-22.	3-9-22.
1.	Number and date						
2.	Fruits taken	Grams	240·5	263·0	250·0	195·0	250·0
	Number of fruits		12	12	12	12	21
3.	Extract made up to c.c.		300	325	275	225	275
4.	Specific gravity of the extract		1·020	1·022	1·024	1·021	1·021
5.	Control—fruits taken	Grams	89·75	91·0	74·8	62·0	62·0
	Number of fruits		4	4	4	4	5
6.	Weight after drying in Grams	Total	29·0	30·0	25·5 (34·1%)	20·0	21·0
	Do.	Husk	13·0	13·0	13·0 (17·38%)	9·5	10·0
7.	Residue from (2)	Grams	61·5	62·5	62·0	48·0	60·0
8.	Volume of distillate, c.c.		207·0	202·0	117·0	152·0	150·0
9.	Sp. Gravity—25°/25°		0·9997	0·9977	0·9942	0·9983	0·9961
10.	Percentage by weight of absolute alcohol		0·21	1·23	3·20	·90	·82
11.	Do. calculated on the weight of dry husk		1·25	6·61	10·2	4·58	2·10