Studies in the Bio-Chemistry of the Mahua Flower.

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By Gilbert J. Fowler, D. SC., F. I. C.

WITH

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Introduction.

The importance of the 'mahua' tree (*Bassia latifolia* and *Bassia longifolia*) has long been recognised, yielding as it does both sugar from the flower and oil from the seed.

It was brought specially to the notice of the senior author of this paper by Mr. G. E. C. Wakefield, then Director General of Revenue, Hyderabad, Deccan, who was in England during the latter part of 1915. It was considered that the flowers might serve as a possible raw material for acetone, then greatly needed for the manufacture of cordite. Experiments in this direction were carried out in the Applied Chemistry Department of the Indian Institute of Science for some months during 1916, but as other material was found more satisfactory, and equally cheap, the possible use of mahua as a raw material for acetone was only partially investigated and attention was directed to its possibilities as a source of industrial alcohol and especially of motor fuel. Here the outlook is most promising. In the Hyderabad distilleries alone, the total possible daily production amounts to 10,000 gallons of ordinary spirit.

In the Report of the British Inter-Departmental Committee on Power Alcohol special mention is made of the evidence given by the Director, Department of Industries and Commerce, Government of H. E. H. the Nizam of Hyderabad, Deccan. Much of the work described in the following pages furnished the biochemical portion of this evidence.

The importance of research work on the resources of India in fermentable sugar is emphasised by the following paragraph on p. 6 of the Committees' Report:—

"We are of opinion that, so far as vegetable sources of raw material for the manufacture of power alcohol are concerned we should rely mainly, if indeed not entirely on increased production in tropical or sub tropical countries."

It is estimated that in the State of Hyderabad more than 21,000 tons per annum of mahua flowers could easily be collected. Quantities also occur in Baroda and Gujerat and also in Mysore. Large distilleries employing mahua as a source of alcohol are in operation in the Bombay Presidency and in Baroda. The necessity for exact scientific information on the bio-chemistry of the mahua flowers is therefore obvious, especially as the published statements are scanty and somewhat contradictory.

A general description and history of *Bassia latifolia* variously referred to as *mahua, mahwa,* or *mowra* is given in Watt's Economic Products of India.

Bassia longifolia flowers also yield a large percentage of sugar as will be seen later.

The leaves of the mahua tree are said to fall in February March or April and to be succeeded in March or April by the flowers. These last for two or three weeks and then begin to fall. The falls take place at night and continue sometimes for a fortnight. When the mahua tree is in bud the ground beneath it is cleared of weeds, sometimes by burning, and the flowers are collected from where they fall.*

The following analyses of the flowers by various authorities may be usefully quoted in detail for the sake of comparison.

Church (*loc cit*)* gives the following figures for air dried flowers :—

Cane sugar	...	...	3·2
Invert „	...	...	52.6
Other matters soluble in water		...	7·2
Cellulose	...	...	2·4
Albuminoids	...	...	2·2
Ash	...	...	4·8
Water lost at 100°C	...	...	15.0
Undetermined	...	...	12·6

* Church. Nature Vol. XXXIII, 1886, p. 343.

Elworthy (J. S. C. I. 1887, p, 21) gives the following figures for flowers from different districts.

	Cane sugar.	Invert sugar.	Dex-trose.	Total sugar.
Hyderabad	17·1	40·0		57·1
Jabalpur	4·6	41·4		46·0
Gujerat	9·6	45·3		54·9
Mirzapur	6·7		43·6	50·3

Analyses made in the Applied Chemistry Department of the Institute of Science showed that the nitrogen content varied from 0·65 to 1·1% being apparently higher in the younger than in the well developed flowers.

The total sugars varied from about 40% in Hyderabad mahua to 60% in a sample from Kaira in North Gujerat.

On the other hand the percentage of disaccharides was higher in Hyderabad mahua varying from 11·0 to 21·7% compared with 2·4 to 11·4 in the Gujerat sample.

The ash content of the flowers varied from 3·6 to 5% and was found to contain appreciable amounts of potash and phosphates.

The statements in regard to the yield per tree vary greatly. Thus Church (*loc cit*) speaks of a single tree yielding as much as 6 to 8 maunds (Bengal maund=82$\frac{1}{4}$ lbs) or even in one case 30 maunds. Watt quotes authorities for anything from 2 to 8 mds. per tree.

The Conservator of Forests in Mysore states in response to a recent inquiry that trees yield up to 2 maunds and come to maturity from seed in 15 years, on the other hand the Director of Industries and Commerce, Hyderabad, Deccan, states that local contractors inform him that each tree yields little more than 18 seers (36 lbs) of flowers and begins to flower after 10 years.

It is evident that the growth and development of the mahua tree may well engage the attention of the economic botanist.

It is hoped that the researches described in the following pages may be useful to future workers.

The work was done under the general direction of the Professor with the assistance from December 1917 to April 1918 of Mrs. R. V. Norris, M. Sc. (Manchester) formerly of the Lister

Institute, London, who had specialised on the subject of yeast fermentations.

The investigation may be divided under the following heads, the accounts of their work having been written by the students concerned and edited by the Professor.

I. Botanical and microscopical examination of mahua flowers, by J. D. Edal Behram and S. Mahdihassan.

II. The enzymes of mahua flowers at different stages of growth, by J. D. Edal Behram.

III. The carbohydrates of mahua flowers, by S. R. Bhate and K. Habib Hassan.

IV. The conditions of fermentation of the sugars of mahua, by N. N. Inuganti and S. R. Bhate.

V. Miscellaneous observations.

VI. Summary and conclusions.

PLATE I.

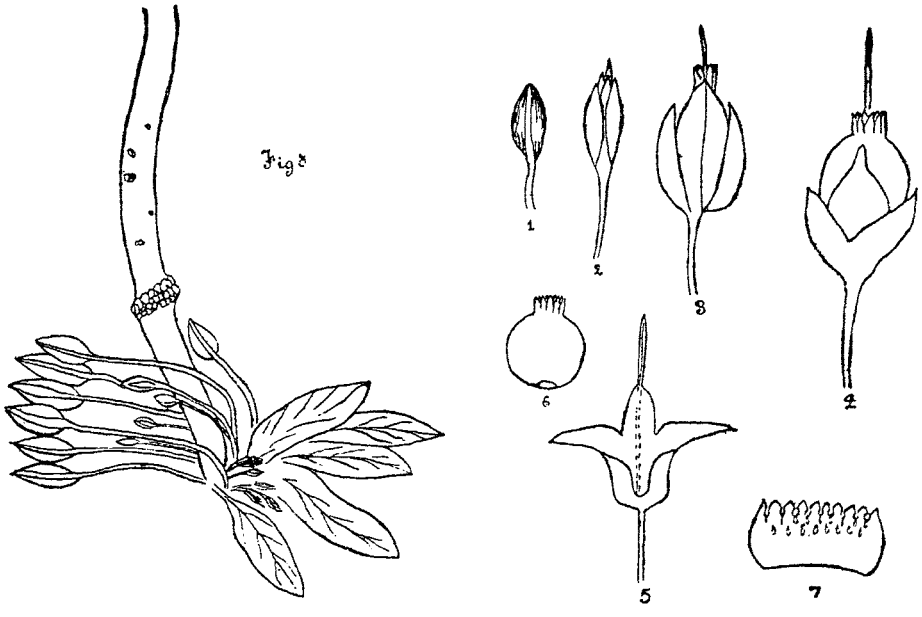

PLATE II.

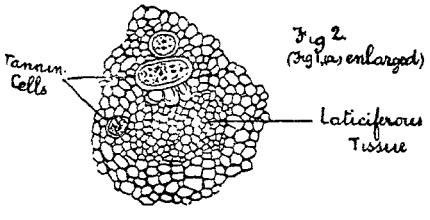

Fig 2.
(Fig 1, as enlarged)

Fig 1

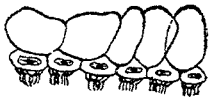

Fig 3.
(Fig 1, (b) enlarged)

I. Botanical and Microscopical Examination of Mahua Flowers.

By J. D. Edal Behram and S. Mahdihassan.

Morphological Observations.

The *Bassia longifolia* trees at Bangalore usually flower about the middle of January. The small leaflets appear first. They are conspicuous by their fresh brown colour and their translucent appearance when seen against light. A little over a week after the appearance of the leaves the flower buds may be noticed. Their flower stocks differ in colour. Evidently *Bassia longifolia* species are not all of the same variety for some trees were found to have a flower stock of a green colour while in others it was pink throughout the entire length. The pink colour is however the characteristic of the typical *longifolia* variety.

For the purpose of study, the life history of the flower was arbitrarily divided into four stages. See Plate I.

1. In the first stage the flower bud is completely closed as illustrated in Fig. 1. The calyx completely encloses the bud which is hard and intact. Soft hairy or villous down is present on the calyx as well as on the flower stock.

2. In the second stage the bud is still closed but the style is seen protruded to about $\frac{1}{4}$ — $\frac{1}{2}$ inch (see Fig. 2). The style may be seen thus a week after the appearance of the bud. The hard and compact flower now becomes softer and more flaccid. The calyx which has been a sort of floral envelope now separates into different sepals. The style at this stage protrudes further to about $\frac{1}{2}$ inch. The anthers are not yet ripe.

3. In the third stage the flower is partially open. The lobes of the corolla are visible. The sepals of the calyx are distinctly separated. The style protrudes to about 2 inches. (See Fig. 3). The corolla at this stage is cream coloured and compared with the following stage is still small in size. Anthers mature and the pollen is shed at this stage.

4. In the fourth stage the flower is considered fully ripe *i. e.* the succulent white corolla is about to be shed (See Fig. 4). The colour of the corolla is less of a porcelain white and is now more translucent than in the previous stage. The calyx is now

completely opened, the sepals are joined only at the base. The corolla is completely exposed and has grown considerably in size. At this stage the corolla drops down with the slightest gust of wind. Fig. 5, shows the flower with the sepals of the calyx and the style, but without the fleshy soft corolla, which is seen by itself in Fig. 6. Fig. 7, shows a corolla dissected. The saw-like edge of the lobes of the corolla represents the petals in a row. Between each two petals lies an anther. There are two rows of anthers as shown in the last figure.

The position of the new leaves in relation to the flowers.

When the flower is in the first stage, the flowers and the leaves appear at first sight to arise from the same whorl, which however is not the case. As the flower approaches maturity *i. e.* about the third stage, the growing point and along with it the portion of the stem, bearing leaves, has advanced about $\frac{3}{4}''$ away from the former joint floral and leaf region. From the very close arrangement of the flowers and the leaves on the stem, one would infer that the leaves might be elaborating the food material and directly feeding the flowers, but that is not so because in some trees under investigation when the flowers had reached practically the third stage, the leaves were in an exceedingly rudimentary condition and very small in size, particularly in the flower variety with the pink petiole (*Bassia longifolia* proper).

The flowers are produced in whorls on the stem. I. appears that the flower first produced, matures first, in other words the whorl close to the growing point matures rather late and consequently flowers in all the four stages above detailed, are met with in a single fascicle. In the same fascicle, a bud and a full blown flower ready to drop its corolla are not at all a rare occurrence.

The flowers grow away from the leaves. In the normal position of the leaves and flowers on the tree, the former grow towards the light, the latter grow downwards, the apices pointing towards the ground. See Plate I, Fig. 8.

After the flowers are produced at a point the apical tissue continues to grow till it produces the requisite number of leaves.

The number of flowers produced at each node is not definite. Quite a number of fascicles were examined and the inference was that the number of flowers commonly met with in each whorl was either 7, 9, 10, 11 or 12, 10 was by far the most common.

The following table gives a general idea as to the relation between the number of whorls, the number of flowers in each whorl and the total number of flowers.

Total number of flowers.	Number of whorls.	Number of flowers in each whorl.
21	3	7
27	3	9
10	1	10
20	2	10
30	3	10
40 not found but possible that it exists.		
50	5	10
33	3	11
24	2	12

10 flowers in each whorl appears to be most frequent. The total number of flowers in a single fascicle was never more that fifty. The figures present interesting variations the results obtained being from a single tree.

MICROCHEMICAL AND HISTOLOGICAL OBSERVATIONS (PLATE II).

Starch. Flowers, in all the four stages, showed an entire absence of starch. Fresh flowers were steeped in alcohol, sections were taken of this dehydrated material. This precaution was taken to avoid enzymic action upon starch. Sections were treated with alcoholic chloral hydrate and stained with iodine when no evidence of starch in the corolla was to be found.

It was however abundant in the pedicles. In the stem it was in abundance in the endodermal region. In stems of an older growth, it was mostly present in the region of the pith.

Tannins. The presence of tannins is of a very general nature. A section of the fresh corolla stained with ferric chloride shows that the epidermal cells on both its surfaces contain tannins. In the corolla, laticiferous tissue is seen well distributed. In the adjoining regions tannin containing cells are also to be found. Fig 1 shows a cross section of a corolla as seen under the low power of the microscope. Fig 2 shows the tannin containing cells adjoining laticiferous tissue. It may be mentioned that tannin in *Bassia longifolia* contains a catechol nucleus. This is shown by the following observations, *viz.*, with ferric salts—a greenish coloration : with bromine water — a yellow precipitate: concentrated H_2SO_4—a dark red coloration occurs at the point

of union. Tannin in the flowers was present in all the four stages. In the stem tannins were also present near the bark as well as also in the region of the pith. They were present in the region of the inflorescence and in the flower stock.

Sugars. The presence of cane-sugar, fructose, glucose and maltose was found in juice crushed from fresh flowers. Sections of flowers preserved in alcohol however showed no presence of crystalline cane-sugar. On treating sections of fresh flowers with phenyl hydrazine hydrochloride and sodium acetate, beautiful crystals of glucosazone and maltosazone were seen under the microsope. It was noted that, while the flower passes from the first to the fourth stage its sweetness increases. This would point to the development of cane-sugar and fructose, as these are sweeter in taste than glucose and maltose. The presence of cane sugar we were not able to confirm microchemically. As fructose unfortunately yields an osazone identical with that of glucose we are unable to say if the increased sweetness of the flower in the fourth stage was due mostly to the formation of fructose.

From the above we infer that in the developing flower the sweet sugars, fructose or cane-sugar, are being formed at the cost of some reserve material which is not sweet but about the nature of which we have no experimental evidence to speak with certainty.

Latex. The composition of the latex of *Bassia* is given by E. Heckel and Schalsden Hauffen (Journal de Pharmacie et de Chimie 1889) as under:—

Water	87·40
Acid formic	trace
Acid acetic	0·50
Insoluble in water 1·66 comprising organic matter	1·405
Ash	0·261
Soluble in alcohol Resin α	2·043
Soluble in acetone Resin β	2·824
Soluble in water Tannin & gum	0·125
Do ash	0·047
Gutta percha	1·803
Ash	3·792
Total	100·000

There is nothing of a carbohydraceous nature present in the latex, as appears from the above analysis. The latex

was examined with the following results. The sap, milky white to start with, coagulates on exposure to air, and becomes flesh coloured. It is tolerably hard at ordinary temperatures but softens when worked with lard and becomes sticky.

A drop of latex from the flower stock directly transferred to a microscope slide and examined after dilution with water shows myriads of small grains. At the first sight they appear as if they were starch grains, as the latex grains show an appearance of hilum and striations characteristic of starch grains. The grains however do not take the blue stain with iodine characteristic of starch.

The latex was next examined for inulin. It was treated with absolute alcohol for two hours and then examined under an oil immersion lens. The grains did not show any resemblance to sphaero-crystals of inulin. A microscopic examination of the latex for tannins after treatment with ferric chloride gave negative results.

Attempts to prepare specific sugar osazones from the latex were not successful.

Fehling's solution was little if at all reduced by a watery emulsion of the latex. The reduction in fact was not appreciable.

With Millon's reagent a brick red coloration was given thus showing the presence of proteins in the latex.

We have mentioned that the flower in the fourth stage has a corolla which has lost its opaque porcelain white appearance and has become instead more or less translucent having an appearance like that of diluted milk. This change in appearance was also accompanied with a change in taste as mentioned above. But the change is also accompanied by change in the latex. The latex or the juice pressed from the flowers in the earlier part of the last stage is much whiter than the juice from the flowers in the final stage. This juice is in comparison more transparent. Haberlandt mentions a similar observation as follows :—

" When the embryo enters upon its period of rest in the ripe seed, the latex becomes transparent ".

The transparency in his case was due to the disappearance of starch in the latex, which cannot be the explanation in our case, as we have ascertained beyond doubt that no starch occurs in the flower even in its very first stage. The sweetening

of the flower, and the change towards the increased transparency of the latex and the flower go intimately together, and we believe the whole phenomenon is to be associated with the function of the main constituent of the latex, which has not yet been ascertained. Latex cells in the flower, *i. e.* corolla are shown in Fig. 2.

Essential oil. When the ripe flowers fall to the ground they are always white, or milky white, but never brown. Only after they have dropped does browning occur. It appears, indeed that browning sets in only when the flower has lost a great deal of its moisture. Browning gradually occurs from the epidermal cells inwards and as the colour becomes deeper a more intense odour is to be noticed. A section of the flower on treatment with alcohol shows that in the epidermal cells there is an essential oil soluble in alcohol. The epidermal cells also contain tannins for they stained well with ferric chloride, see Fig. 3.

II. The Enzymes of Mahua Flowers at Different Stages of Growth.

By. J. D. Edal Behram.

A systematic examination of the flowers was made at various stages to determine the presence of the enzymes and their nature.

Method of preparing the extracts—25 gms. of freshly plucked flowers were ground to a pulp in a Wedgewood mortar and 5 gms. pure sand (treated with hydrochloric acid and washed completely free of acid) were thoroughly worked up with the pulp so as to rupture the cells. 50 ccs. of water containing 5 ccs. saturated thymol water as antiseptic were now incorporated with the mixture and the whole left for three hours. The extract was now strained through cloth and used for determining the presence of various enzymes.

Carbohydrases.

Amylase — 25 ccs. of a 0·5% soluble starch paste were placed in four sterile test tubes and different quantities of the extract were added to the paste as under.

Tube (a) was kept as control after adding to it 10 ccs. distilled water; to

Tube (b) 5 ccs. boiled extract plus 5 ccs. distilled water were added.

Tube (c) was kept with 5 ccs. unboiled extract plus 5 ccs. distilled water, and

Tube (d) was kept with 10 ccs. unboiled extract. The total volume in each of the four tubes was 35 ccs.

The tubes were now kept in a water bath at 40°C for three hours. One cc. out of each of the tubes (a) (b) (c) and (d) was placed in a separate Nessler glass and brought to the 50 cc. mark by addition of distilled water, and a drop of N/10 iodine carefully added from a 1 cc. standard pipette to each. The coloration imparted to starch was now compared on a Nesslerizing stand :—

(a) gave an intense blue coloration,

(b) gave an intense blue coloration equal to (a).

(c) a reddish coloration, and

(d) no coloration at all.

It is evident therefore that amylase was hydrolyzing starch.

Amylase was looked for in all the four stages of the flower and even in the dropped corolla.

Flowers in all the four stages were picked out from a single bunch, their enzymes extracted and examined for amylase as detailed above, with starch paste.

Tube (1) 1st stage extract 5 ccs. plus 10 ccs. of 0·5% starch paste.

(2) 2nd stage extract 5 ccs. plus 10 ccs. of 0·5% starch paste.

(3) 3rd stage extract 5 ccs plus 10 ccs. of 0·5% starch paste.

(4) 4th stage extract 5 ccs. plus 10 ccs. of 0·5% starch paste.

(5) Extract from dropped corolla 5 ccs. plus 10 ccs. of 0·5% starch paste.

These tubes were incubated at 40°C for one hour and a half and tested for starch with iodine with the following results by the method already detailed.

(1) No blue coloration
(2) Very slightly blueish red
(3) Blue coloration
(4) Intense blue, equal to the control
(5) Ripe corolla extract as blue as the control.

It is thus evident that the greatest and the most powerful quantity of amylase is present in the first stage. In stage 2 it is present but not to an equal amount. In the third stage it is disappearing and in stage 4 it is entirely absent, so also in the dropped corolla.

Inulase—The next enzyme investigated was inulase. 25 ccs. of a one per cent solution of inulin were introduced into three sterile test tubes.

(1) was kept as control with 5 ccs. distilled water; to
(2) 5 ccs. of the boiled extract were added, and to
(3) 5 ccs. of the unboiled extract were added.

All the tubes were incubated at 37°C for 24 hours. The liquid from tube (3) was tested by Selivanoff's special test for fructose. (This consists in adding a few crystals of resorcinol to a mixture of equal parts of hydrochloric acid and water. A very small quantity of fructose solution is now added and the whole gently heated. The solution becomes red in colour and deposits a brownish red precipitate which dissolves in alcohol giving a red solution. ω-Hydroxy methyl furfural is formed by the action of the acid on fructose and combines with the resorcinol giving the red pigment). A red coloration was not immediately obtained, as is obtained on heating fructose. A prolonged boiling however gave a red coloration. This was due to the gradual formation of fructose from inulin under the action of hydrochloric acid, as was next confirmed by starting from actua inulin which under similar conditions gave a red coloration.

To confirm that no fructose was formed from inulin by the action of inulase, the preparation of fructosazone from a fresh lot of the liquid in tube (3) was tried. No osazone was obtained.

In case 37°C might be too high a temperature for the action of inulase the tubes were in another experiment kept at the room temperature 24°C—28°C for 72 hours with thymol as antiseptic. The preparation of the osazone was again attempted but with negative results.

A micro-chemical examination was also made with a view to see whether inulin was present in the flower. Inulin could not be detected and naturally along with it, its associated enzyme inulase, is also absent.

Maltase—Twenty ccs. of a one per cent solution of pure maltose were pipetted into three sterile test tubes. Five ccs. of distilled water were added to No. I tube and kept as control. 5 ccs. of boiled enzyme extract were added to No. II and 5 ccs. of unboiled extract were added to No. III. The three tubes were incubated for 24 hours at 37°C.

An osazone was prepared from the liquid in each of the three tubes.

Tubes I and II gave a pure crop of maltosazone, while III showed sheaves of needles of glucosazone which was the first to appear in abundant quantity. The mother liquor on concentration, and after filtering off the glucosozone deposited further quantities of glucosazone along with a little maltosazone.

It is thus evident that maltase is present, Maltase was found to be present in all the four stages, in what amounts however it has not been yet determined.

In this connection it was interesting to confirm the presence of maltose in the first stage flower.

Fifty gms. of first stage flowers were crushed to pulp in a Wedgewood mortar and allowed to extract with 100 ccs. cold distilled water for 2 hours. The extract was now strained through cloth and pure kaolin added to clarify and then filtered The filtered extract was now reduced to 25 ccs. by evaporating on a water bath and an osazone prepared. A pure crop of maltosazone resulted showing that maltose is present even in the unripe bud.

A hot water extract from the same pulp after exhausting in the cold gave a glucosazone along with maltosazone, the latter in comparatively very small amounts.

Invertase. With a view to avoid as far as possible the introduction of reducing substances which might occur if merely a cloth strained extract were used, the extract in the investigation of this enzyme was passed through a filter candle. The clear extract obtained in this manner was utilized immediately for investigation purposes.

A five per cent cane sugar solution was prepared (the distilled water used for making the solution contained 10 ccs. saturated thymol water as antiseptic for every 90 ccs. of distilled water) and 45 ccs. of this solution was pipetted into separate test tubes and 5 ccs. of the unboiled extract added to each. Two of these tubes were immediately transferred to a boiling water bath, kept in the bath for an hour and cooled. The volume was made up to 50 ccs. and titrated with Fehling's solution.

As the beginning of the experiment 2 ccs. Fehling's required for reduction 35 ccs. of the mixture of cane suger solution and enzyme extract.

At beginning of experiment	...	35·0 cc.
After 24 hours	...	19·7 cc.
After 48 hours	...	10·6 cc.
After 72 hours	...	6·0 cc.
After 96 hours	...	5·5 cc.

With the passage of time the cane sugar under the influence of invertase changes into the reducing sugars glucose and fructose and thus a lesser and lesser quantity is necessary for the complete reduction of 2 ccs. Fehling's solution. Thus the presence of invertase is evident.

Invertase was similarly looked for in all the stages and found present. About the fourth stage however it seems to be decreasing.

GLUCOSIDASES.

It appeared likely that the sugars would be present in part as glucosides, and the presence of glucosidases such as emulsin was sought for.

Emulsin. Fifty gms. of first stage flowers were ground to a pulp in a mortar and 10 gms. pure sterile sand added and the whole again thoroughly ground together so as to rupture the cells. A two percent solution of amygdalin was next prepared and 25 ccs. of this solution were pipetted out into four test tubes.

(1) was kept as control

(2) Five gms. of the pulp prepared as above were kept in a steam oven for three hours and then added to tube (2)

(3) To this tube 5 gms. of the ordinary pulp were added

(4) Ten gms. of the pulp were added.

Picric acid paper* was now suspended in all the four test tubes by means of strings taking care not to allow the paper to touch the liquid; the test tubes were all corked, and kept at room temperature. A brick red coloration began to appear on the paper owing to the evolution of hydrocyanic acid gas from amygdalin under the influence of the enzyme emulsin.

Tube (1) no change.

(2) no change.

(3) paper distinctly brick red.

(4) intensity of color nearly double that of (3), thus showing a distinct evidence of the presence of emulsin in stage one.

* Picric acid paper was prepared by steeping strips of filter paper in a solution of one gm. picric acid, ten gms. sodium carbonate and hundred ccs. water.

The corolla extracts in all the four stages were next examined for the presence of emulsin, as follows :—

Experiment :—

1st stage 5 ccs. extract plus 25 ccs. 2% amygdalin solution
2nd „ „ „ „ „ „ „ „ „ „
3rd „ „ „ „ „ „ „ „ „ „
4th „ „ „ „ „ „ „ „ „ „

In three hours time the filter paper strip in the first stage tube, developed a brick red tinge, the second stage experiment showed the same evidence, whereas the third and fourth stage extracts showed no signs of activity.

After about six hours the first and second stage extracts developed a distinct brick red coloration, the coloration in the former being much more pronounced than in the latter, whereas not the slightest sign of coloration was presented by either three or four.

After 24 and even 48 hours the third and fourth stage extracts recorded the same evidence, thus showing that emulsin had disappeared about the third stage, and probably along with it its corresponding substrate glucosides In the first stage flower however the emulsin seems to be present in a greater quantity than that in the second stage, from the fact that the coloration in the former developes much faster than in the latter and by the end of the experimental period is much more intense.

The presence of emulsin shows that in the first and second stages glucosides are present. They however probably disappear about the third stage for in that stage emulsin is absent.

Oxidases.

Along with the carbohydrases and glucosidases, the oxidases were also studied.

Catalase—In making a determination of the catalase content of the mahua flower a measured volume of the extract prepared as detailed above was introduced into a fat extraction flask and a tap funnel containing 25 ccs. of a 3 volume solution of hydrogen peroxide was inserted into a cork of the flask, and a bent delivery tube passing through the neck of the flask joined the flask to a eudiometer, the flask and the eudiometer being kept in a Hearson cool incubator. As soon as the apparatus attained the experimental temperature (cool incubator at 20°C) the hydrogen peroxide was run into the flask and the displaced air allowed to pass out by means of another tube fitted into the cork of the

flask and ending in a glass tap. The tap was immediately closed, the extract mixed by shaking. The volume of oxygen evolved in a two hour period taking readings at intervals of 15 minutes was recorded. A control with boiled extract was also kept side by side. The results obtained in an experiment were as under :—

	Oxygen evolved in ccs.	
	Experiment	Control
To start with ...	0	0
After 15 minutes ...	12·00	0
„ 30 „ ...	17·00	0
„ 45 „ ...	19·00	0
„ 60 „ ...	20·50	0
„ 75 „ ...	21·50	0
„ 90 „ ...	22·00	0
„ 105 „ ..	22·50	0
„ 120 „ ...	22·50	0

The total oxygen evolved in a two hour period was 22·5 ccs. There was sufficient hydrogen peroxide left over in the flask for further evolution of oxygen but apparently the activity of the enzyme had stopped. Catalase was found present in all the stages examined and in practically identical amounts.

Oxidase. The method adopted in the preparation of the extract for the investigation of oxidase was slightly different. The same process as detailed above for the preparation of extracts was followed but with one addition, *viz*, along with sand five gms of hide powder (pure for analysis) were added to precipitate the tannins. The extract thus rendered free from tannins was utilized for the investigation of oxidase.

This extract gave a distinct blue reaction with guaiacum alone and therefore contains the full oxidase complement oxygenase and peroxidase.

A qualitative examination showed that at all the four stages of the flower the full oxidase complement was present. The author has not examined whether the oxidase content varied in the different stages or kept the same in amount.

A rather rough and ready determination showed that the oxidase content of the corolla lobes was increasing after the corolla had naturally dropped from the flower. This was determined as follows. Corolla lobes from 100 ripe fourth stage

flowers and those from 100 corollas naturally dropped from the tree, were washed free of adhering anthers and grit, the lobes were next freed from excess moisture by pressing between folds of filter paper and finally completely dried by keeping in a dessicator for two days 0·25 gms. of each were separately weighed, ground in a mortar and 25 ccs. distilled water incorporated, and allowed to stand one hour. 10 ccs. from each was now pipetted into two separate test tubes and 20 ccs. of two per cent guaiacum tincture added to each. The blue flocculent mass that came down was dissolved in 10 ccs. of 95 per cent alcohol and filtered clear. The clear filtrate was next transferred to Nessler glasses and their tints matched. It appeared that the tint of 40 ccs. of the filtrate from the fourth stage corolla lobes matched exactly with that of 30 ccs. filtrate from the lobes of the dropped corolla. It is thus evident that the oxidase content of the corolla lobes increases after the corolla is detached from the flower. The original cream coloured corolla now begins to get brown starting from the point of attachment of the lobes to the body of the corolla. This gradual browning progresses so far with the passage of time that the original cream color of the corolla now becomes earthy black, and with this browning and subsequent blackening the total sugar content of the dropped corolla appreciably falls. To cite an instance, the total sugar content in the fourth stage (ready to drop) corolla was 74·2 per cent to start with. In about three and a half months time it fell as low as 50·8 per cent. The dropped flowers were kept between two clock glasses in the laboratory. The sugar content was calculated on the dry matter. The author has not worked out the progressive fall in sugar content with the passage of time but it is a problem worth tackling as it may reveal storage conditions under which it may be possible to prevent such a loss of these valuable fermentable sugars.

This increase in the oxidase content above referred to, after the vital processes have either stopped or are at a low level has also been observed by Suzuki and Bunzell.*

" Since upon the death of the protoplasm, oxidases act without the restraints to which they are subject during its life, it might well be supposed that conditions unfavourable to the normal metabolism of the cell might result in an increased oxidase activity. It has been observed that when mulberry trees were cut back too frequently, an abnormal yellow color and crinkled appearance resulted in the leaves Suzuki investigating this found that an excessive production of oxidases had taken place in these areas."

*Bulletin No. 298, U. S. A. Department of Agriculture.

The physiological function of the plant oxidases has been investigated by Palladin (Ber. d. Deut. Bot. Gesell; 1908 XXVI, 378, 379). "It is a matter of common observation that leaves when killed by frost or severance from the tree frequently assume a brown, black or red color; Palladin has brought forward the view that the respiration of a substance such as glucose is hydrolytic oxidation, whereby the carbon is oxidised anaerobically to carbon dioxide, and the hydrogen thus set free, combines with a respiratory pigment, reducing it to a colorless chromogen. In the following aerobic stage oxygen is absorbed, with the production of water and the pigment. These processes are shown in the following equation."

I Anaerobic stage :—

$C_6H_{12}O_6$	+	$6H_2O$	+	$12R$	=	$6CO_2$	+	$12RH_2$
Glucose	+	Water	+	Respiratory pigment	=	Carbon dioxide	+	Chromogen

II Aerobic stage :—

$12RH_2$	+	$6O_2$	=	$12H_2O$	+	$12R$
Chromogen	+	Oxygen	=	Water	+	Pigment

This probably explains the browning and final blackening of the dropped corolla.

Cellulose Dissolving Enzymes.

Cytase. 15 ccs. of the first stage enzyme extract were put into a sterile Petri dish. Another lot of 15 ccs. killed by boiling were put into a second Petri dish. Thin sections of potato (peel and pulp combined) were taken with a razor and examined under the microscope. Only those sections were selected which had their cell walls intact. The selected sections were saturated for half an hour in thymol water and were then transferred to the petri dishes containing the enzyme extracts, and examined from day to day under the microscope. The petri dishes were incubated at 37°C. About the fifth day the cell walls of the potato sections in the unboiled extract showed some signs of wearing away. The changes however were slight in a fairly long period and again rather difficult to verify. Taking into consideration the tendency of enzyme solutions to deteriorate with time, and also the difficulty of keeping the experiment sterile, it is not possible definitely to assert the presence of cytase at this stage.

Pectase was similarly looked for by placing sections of banana peel in the boiled and unboiled first stage enzyme extracts but no definite and decisive evidence as to the presence of that enzyme was forthcoming.

The foregoing results regarding the specific enzymes present in the flower at its different stages of growth may be summarized in the following table :—

Name and class	Stage I.	Stage II.	Stage III.	Stage IV.
(*A*) *Carbohydrases.*				
(1) Amylase	present	present	slight, disappearing	absent
(2) Inulase	absent	absent	absent	absent
(3) Maltase	present	present	present	present
(4) Invertase (sucrase)	present	present	present	slight
(*B*) *Glucosidases.*				
(1) Emulsin	present	present	absent	absent
(2) Invertase	present	present	present	slight
(C) *Oxidases.*				
(1) Catalase	present	present	present	present
(2) Oxidase	present	present	present	present

It thus appears that amylase disappears as soon as the flower opens. The same is the case with emulsin. Invertase decreases about the fourth stage. It is included both as a carbohydrase and also as a glucosidase, which it may be termed on the assumption that cane-sugar has a glucosidic structure. Maltase, catalase and oxidase are present throughout. The presence of pectase is indefinite like that of cytase.

Enzymes of the Leaf.

Having studied the enzymes of the flower the leaves were next examined for their enzyme content.

Fifteen gms. of very young leaves bearing red pigment and plucked from the immediate neighbourhood of the flowers were ground to a pulp in a mortar and allowed to stand three hours with 30 ccs. water containing thymol as antiseptic.

Oxidase and catalase were looked for exactly as in the flower and found present.

Amylase was found to be present. So also emulsin.

The remaining enzymes were not looked for but the foregoing shows that the leaf and the flower contain practically the same enzymes.

Enzymes of the Latex.

An examination of the enzymes of the latex shows the presence of a powerful oxidase which gives an intense blue coloration immediately on the addition of guaiacum tincture, without the addition of hydrogen peroxide, which shows that a full oxidase complement is present.

Catalase is absent. The carbohydrases were not looked for as the analysis and examination previously mentioned show that the latex does not contain the suitable substrate for these enzymes.

III. The Carbohydrates of Mahua Flowers.

by S. R. Bhate and K. Habib Hassan.

As stated in the introduction, the various analyses hitherto published of the sugar content of mahua flowers show considerable variation according to the origin of the flowers and their age. Moreover no further differentiation is made beyond the statement of the percentage of non-reducing sugar as cane sugar and reducing sugar as invert sugar.

In the present investigation an attempt was made to distinguish a greater variety of sugars and also to determine if possible the changes in sugar content corresponding to the different stages in development of the flowers. These stages were the same as described in the introduction.

Methods of analysis.

The methods of analysis adopted may be briefly described as follows, the more important determinations being made independently by both authors:

Moisture The corollas of 25 sample buds were taken, weighed between two watch glasses and dried in the steam oven for 5 to 6 hours at 99°C and further dried in a vacuum dessicator to a constant weight.

Extraction. The sugar can be readily dissolved out from the flowers by hot or cold water. Both methods were adopted although there is some possibility of inversion taking place in each case, due, with hot water, to any acids which may be present, and, with cold water, to the action of enzymes. In practice little difference was observed in the analytical results from both methods. 100—125 buds were taken and crushed to a mass in a mortar. Four extracts were taken. With cold water a little ammonia was added with a view to inhibit enzyme action.

Sugar Estimation. The extract though clear is of a brown colour too deep for its angle of rotation to be read in the polarimeter, consequently a decolourising agent has to be employed. In spite of the fact that basic lead acetate slightly affects the rotation of fructose it is on the whole the most satisfactory reagent. Excess of basic lead acetate is added and after standing 2 or 3 minutes the excess of lead is precipitated by sodium phosphate and the clear and colourless solution taken for sugar estimations.

A portion of the clarified solution was examined directly in the polarimeter for specific rotatory power and in a second portion the cupric oxide reducing power was determined.

Another portion was inverted by Clerget's method, 10 cc. of strong hydrochloric acid being added to 100 cc. of the solution, which is then heated from 67°C to 70°C for 7 to 10 minutes and cooled immediately. A portion of the inverted solution was examined polarimetrically in a 2·2 decimeter tube and the other was neutralised with sodium bicarbonate for finding the cupric oxide reducing power.

The cupric oxide reducing power was determined gravimetrically, using a Gooch crucible with an asbestos filter to collect the cuprous oxide. This was ignited and weighed as cupric oxide.

Calculations. From the change of rotation between the direct reading and the reading of the solution after inversion the percentage of non-reducing sugar as cane sugar was found by taking 1·73° as the change in rotation for 1% solution of cane sugar at 25°C.

From the percentage of cane sugar the rotation due to cane sugar was calculated, 1·33° being taken as the rotation for 1% solution of cane sugar.

This was deducted from the total direct rotation to get the rotation due to dextrose, levulose and other sugars. As the sugars other than dextrose and levulose are of less frequent occurrence and have a very high rotatory power they may provisionally be ignored and the whole dextro rotatory sugar be assumed to be dextrose.

The cupric oxide reducing power for 100 cc. of solution was then calculated from the cupric oxide reducing power determined actually.

Two equations were obtained as follows :—

$$1{\cdot}05\,X - 1{\cdot}763\,Y = \text{total rotation} - \text{rotation due to cane sugar.}$$

$$2{\cdot}54\,X + 2{\cdot}36\,Y = \text{cupric oxide reducing power of 100 cc.}$$

1 05° and 1·763° are the rotations due to 1% solutions of dextrose and levulose respectively. 2·54 grams and 2·36 grams

of CuO are obtained from the Fehling solution by 1 gram of dextrose and levulose respectively.

The solution of these equations gives the percentage of dextrose and levulose.

As already noted, sugars other than dextrose may be present but dextrose has a specific rotation of 52·5° whereas other dextro-rotatory sugars have the following specific rotations :—

Maltose	plus	140°
Raffinose	,,	104·5°
Galactose	,,	83·88°

If any of these sugars are present it will be in very small amounts for if their proportion is greater it means that a greater proportion of levulose will also be present to counteract the high dextro – rotation.

The values for X and Y in the above equations therefore indicate the maximum of dextrose and the minimum amount of levulose.

Non-reducing sugar was, as a first approximation, assumed to be cane-sugar. There are sugars like maltose and raffinose which have little or no reducing power before inversion but have a greater power after inversion.

The presence and proportion of these sugars can only be determined by the preparation and examination of osazones.

The presence of pentoses will also affect the calculations for dextrose, levulose and cane-sugar.

As a first approximation however the results may be tabulated as follows :—

Flowers.	Dextrose	Levulose	Cane sugar	Total sugar	Total invert sugar
1st stage	(11·35)		3·37	14·72	14·9
2nd ,,	6·76	11·68	3·60	22·04	22 2
3rd ,,	7·42	17·50	11·38	36·30	36·9
4th ,,	18·64	20·12	28·80	67·56	69·0
5th ,, flower after storage.	19·64	23·30	18·75	61·69	
Old syrup from mahua flowers, containing 23·5% moisture	35·61	35·25	5·5	76·36	

A number of analyses were made of flowers in the fourth stage from day to day giving the following results, which serve to show the limits of variation.

Cane-sugar	28·92	31·47	34·24
Levulose	22·4	19·66	17·43
Dextrose	16·4	16·0	15·81

It is clear from these results that the cane-sugar goes on increasing as the flowers develope and is greatest when the corollas are about to drop.

On keeping, the percentage of cane-sugar rapidly decreases as is shown by the lower percentage in stored flowers due probably to partial fermentation as well as to enzyme action. Finally in old mahua syrup the percentage of cane-sugar or non-reducing sugars is small.

Preparation of Osazones.

Osazones were prepared from the syrup obtained by evaporating the extract from fresh flowers in the 4th stage. On warming with phenyl hydrazine and acetic acid a good crop of crystals was obtained which were identified as glucosazone by their appearance under the microscope. The mother liquor was further heated for an hour and a second crop was obtained which along with glucosazone showed some other crystals also. Maltosazone was suspected and an attempt to separate it out was made by boiling the whole precipitate in water and filtering it hot. On allowing the filtrate to cool a precipitate came down. Maltosazone is soluble in boiling water and on examination the crystals showed an appearance resembling that of maltosazone as figured in text books Some maltosazone was prepared from pure maltose and the crystals found to be exactly similar to those under examination.

Both osazones were further confirmed by melting point determinations. The melting point of glucosazone was found to be 211° to 212°C using a naked flame to heat the sulphuric acid bath with a rise of temperature of 12° per minute. With the sulphuric acid heated on a sand bath with a rise of 2° to 3° per minute the melting point was 190° to 191°C. The melting point of maltosazone under the former conditions was found to be 200°C.

A rough idea of the quantity of maltose could be obtained by weighing out the precipitates of glucosazone and maltosazone The maltosazone precipitate weighed about a fifth

of the weight of glucosazone. As dextrose and levulose together in the stage examined were 43% the percentage of maltose comes to 8 or 9%. The cane-sugar in the sample was calculated to be 18%.

Maltose can be estimated quantitatively by fermenting two portions (1) by yeasts like *S. marxianus*, *S. exiguus* and *S. anomalus* which do not ferment maltose and (2) by ordinary brewery yeast. The copper oxide reducing power of the fermented solutions would give by difference the quantity of maltose. As these yeasts were not available, a quantitative estimation could not be made.

Meanwhile a method of estimating maltose by means of invertase was tried on the assumption that the invertase used would only affect cane-sugar. The invertase was obtained by treating brewery yeast with acetone for 2 minutes (to kill the yeast without killing the invertase contained in it), and then with water. Equal quantities of the invertase solution were added to equal volumes of a three per cent cane-sugar and an equally diluted mahua sugar solution. When all the cane-sugar was found to be inverted the cupric oxide reducing power for the mahua solution was determined. Another portion of the mahua solution was inverted by Clerget's process, neutralised and the cupric oxide reducing power determined. The difference between the two readings gave the reducing power due to maltose The results thus arrived at showed the presence of 6 per cent of maltose.

The decoction of flowers in the fourth stage was also subjected to complete fermentation with mahua yeast. Estimation of total sugar before and after fermentation showed an unfermented and presumably unfermentable residue of from 4 to 6%. An endeavour was therefore made to determine the presence of *pentoses*.

Estimation of pentoses. In the absence of phloroglucinol the precipitation by phenyl hydrazine acetate of the furfurol obtained by the hydrolysis of pentoses suggested itself as the best available method of estimation. For this purpose the solution was distilled with hydrochloric acid of 1·06 sp. g. and the distillate collected in 30 cc. lots; after each collection a corresponding quantity of acid was added through a dropping funnel. The distillation was continued till no red colour indicative of furfurol appeared on testing the distillate with aniline acetate.

The acid distillate was made alkaline by addition of sodium carbonate in slight excess and then acidified with acetic acid and made up to a known volume. An aliquot portion was precipitated with phenyl hydrazine as a hydrazone, the precipitation being hastened by stirring. The precipitate was filtered in a Gooch crucible and dried in vacuo at 60—70°C or in a slow current of dry air at this temperature. The liquid filtered from the precipitate was tested with aniline acetate to see that all furfurol had been converted into the hydrazone.

From the weight of the dried precipitate, the weight of furfurol and thence of the pentoses present can be calculated, and was determined as 2·04%.

Other carbohydrates. No starch or dextrin could be found in any part of the flower. A few isolated grains of starch sometimes present can be accounted for as washed up grains from the guard cells of the stoma.

Cellulose was estimated by both the following methods which each gave about 4 per cent of the dry matter as cellulose.

A solution of 30 grams powdered potasium chlorate was prepared in 520 cc. of cold nitric acid (1·1 sp. g.) the flowers suspended in this at a temperature of 20°C till the whole mass was quite white. This took about 10 days.

The flowers after dissolving out the sugars were treated with a large quantity of Schweitzer's reagent until the solution was complete and then on acidification the cellulose was precipitated as a floeculent precipitate and weighed.

Summary of Observations.

The presence of the following carbohydrates has been definitely ascertained:—

Glucose (dextrose), fructose (levulose), maltose, sucrose (cane-sugar), pentose, cellulose.

The results of the analyses of the flowers at various stages of growth show that in the growing stage fructose is always present in greater amount than glucose. In the fourth stage the quantities approximate but do not become equal. In the fourth stage also the percentage of sucrose attains its maximum, but this soon falls off as the flowers are kept in store.

IV. The Conditions of Fermentation of the Sugars of Mahua.

By N. N. Inuganti and S. R. Bhate.

The following experiments were carried out with the object of determining the conditions for obtaining the highest possible yield of alcohol from mahua flowers.

Natural fermentation. A number of fermentations were conducted under natural conditions *i. e.* with the natural yeast and other organisms present in the flowers as they reached the laboratory. 100 grams of flowers were used for each experiment and the conditions such as temperature, proportion of water added, method of using the flower, whether crushed or uncrushed or merely in the form of extract, were varied in different experiments. In no case was the yield of alcohol in these experiments found to be more than 60% of the theoretical.

Preparation of pure cultures of yeast. Hansen's method of separation was used. Mixed colonies from flower washings were grown on plates of mahua extract and agar, a speck from a colony was removed with the end of a sterile platinum wire and inoculated into 1 cc. of sterile water. A loop was then taken on a squared slide and the number of cells counted under the microscope. Having found eight cells on the loop it was diluted eight times with sterile water so that each loopful of the diluted liquid may be supposed to contain a single cell if the liquid has been well shaken.

From these, four plate cultures were made by inoculating single loops on to mahua agar when three colonies only appeared on two plates. Each colony was found when examined under the microscope to contain a pure growth of a single variety. Two species were identified, which were isolated and preserved in mahua agar tubes. One species was *Saccharomyces cerevisiae* and the other *Saccharomyces ellipsoideus.*

Pure culture fermentations. Experiments were first made with a single variety of different kinds of yeast (Table I p. 112 Exps. 1–6) and then with a mixture of the above mentioned two varieties of yeasts a number of fermentations were carried on, the conditions and results of which are summarised in the table (p. 112 Exps. 7—16).

From these and the experiments previously mentioned it may be concluded that the most favourable conditions for a

good yield are (1) inoculation with pure cultures under sterile conditions (2) addition of substances such as ammonium phosphate and sulphuric acid in small quantities (3) addition of spent wash which forms a good food and nutrient to yeast (4) a temperature of 30° to 35°C. Incidentally it was also found that aeration at the beginning of the fermentation and occasional stirring were helpful to the process.

Not more than 100 grams of flowers were used in each of these experiments.

Larger Scale Experiments.

The mahua used for these experiments had been in store for a very long time and the percentage of total sugar was estimated to be only 31·2%. At the time it was the only material readily available for experiments on something more than a laboratory scale. It was however important to determine whether the results obtained in the laboratory experiments could be repeated with larger quantities of materials under conditions approaching those of the distillery.

The material was placed in a steam jacketed still provided with a screw cap through which the contents of the still after sterilisation could be inoculated with a pure culture of yeast. After fermentation was complete the alcohol formed was distilled off as an aqueous distillate and the actual quantity of absolute alcohol obtained found by determining the specific gravity of successive portions of distillate at 60°F. The following are the details of the experiments:—

Experiment I. Still of 10 gallons capacity used.

25 lbs of the flowers were crushed and mixed with about 10 gallons of water, sterilised well and cooled. To the cooled mash 4 drachms of commercial sulphuric acid and 4 ozs crude ammonium phosphate were added. The whole was inoculated with a litre of fermented mahua solution containing a pure culture of *Saccharomyces ellipsoideus*

The following are the laboratory notes of the experiment :—

Inoculated on March 21st (1918) at about 11 a. m.
Gasification started on 23rd (no stirring).
Gasification continued up to the 26th noon.
Distillation carried out on 27th morning.

On distillation the several distillates obtained were :—

		Volume of absolute alcohol.
1.	1000 cc. of ·830 (hydrometer) or 91% absolute alcohol. =	910 cc.
2.	425 cc. of ·835 (hyd.) or 89% absolute alcohol. =	378 cc.
3.	750 cc. of ·875 (hyd.) or 76% absolute alcohol. =	570 cc.
4.	425 cc. of ·915 (hyd.) or 59% absolute alcohol. =	250 cc.
		2108 cc.

The theoretical yield of absolute alcohol from 25 lbs of mahua containing 31·2% of sugar may be calculated as 2250 cc. so that even with unsatisfactory raw material about 93% of the theoretical yield is obtained. The spentwash obtained after distillation was found to contain traces of unfermented sugar.

Experiment II. Still of 100 gallons capacity used, provided with stirring gear.

92 lbs of flowers were crushed and mixed with 32 gallons of water. This was sterilised and cooled and 8 drachms of commercial sulphuric acid and half a pound crude ammonium phosphate were added. The mixture was then inoculated with 2 liters of fermented mahua containing pure cultures of *saccharomyces ellipsoideus*. As the inoculation took place before the distillation of the first brew, no spent wash was available as additional yeast food.

The following are the laboratory notes :—

Inoculated 10 a. m., March 27th 1918.

After inoculation the mass was stirred from time to time viz on the evening of the 27th and on the three following days.

Gasification started on the 29th about 10 o'clock.

Do much diminished on the 80th.

Left undisturbed till 1st April.

Distillation carried out on morning of April 1st.

The large volume of distillate obtained was mainly 50-60 U. P

This was fractionated in the small still used for experiment I. The following fractions were obtained:—

1.	5150 cc. of	·827	s. g,	*i. e.*	92% or absolute	1978 cc.
2.	2425 cc.	·835	,,	,,	89% ,,	2158 cc
3.	2385 cc.	·840	,,	,,	89% ,,	2046 cc.
4.	1295 cc.	·845	,,	,,	86% ,,	1113 cc.
						7295 cc.

The actual theoretical yield is 7950 cc. the percentage yield being therefore 92%. As before the spent wash contained traces of unfermented sugar. Consequently it is possible that a somewhat higher yield might have been obtained if spent wash had been added to the brew.

It is thus evident from these trials done under semi-technical conditions that yields equal to those obtained in the laboratory may be expected in the distillery when the process is carried out under scientific conditions.

It will be seen from experiment No. 2 that the amount of ammonium phosphate and sulphuric acid is not proportionately increased as compared with experiment No. 1, showing that the quantity added may have been more than was actually required for experiment No. 1. It is of importance to determine the minimum quantity of these substances needed to produce the greatest yield of alcohol.

It is satisfactory to learn from Mr. Nagarkatti, Assistant to the Director of Industries, Hyderabad that the results given in the present paper have actually been obtained on large scale pure culture fermentation trials carried out in Baroda from 1908—11 but not published.

V. Miscellaneous Observations.

Essential Oil.

The peculiar unpleasant odour of mahua and of spirit made from mahua flowers is due to an essential oil. As the smell varied with flowers from different sources and of different species an attempt was made by Mr. Edal Behram to determine the essential oil in two varieties of the flower.

The method of estimation was similar to that used by Mann for quantitatively comparing the flavour of various teas (Mann 'Fermentation of tea' part II, p. 4, Indian Tea Association) *viz.* the determination of the amount of oxygen required completely to oxidise the essential oil. Twelve grams of freshly plucked 'mahua' flower corollas in which moisture was separately determined were added to 750 cc. of distilled water kept boiling in a round bottomed boiling flask. The flask was immediately connected to a condenser and steam blown through the flask which was also heated so as to obtain one litre distillate in about 2 hours. The distillate was collected in successive portions, four of 50 cc., five of 100 cc. and one of 250 cc. To each of these portions 10 cc. of sulphuric acid (1 : 5) and 50 cc. of potassium permanganate (1 cc. $KMno_4$ = 0·1 Mgm. available oxygen) were added. Each lot was allowed to stand two hours in the incubator at 37°C, cooled under the tap, and the excess of permanganate titrated with thiosulphate and starch.

In the case of a sample of *Bassia longifolia* having large flowers, a pink style and a powerful odour the total oxygen absorbed under the above experimental conditions was 0·212%, whereas with a small flower a variety of *Bassia longifolia* which had a less pronounced odour the total oxygen absorbed was 0·105%.

It is evident that wide variations in the percentage of essential oil exist and it is important therefore to study further the conditions of its occurrence and formation. It would appear to become more apparent with the browning of the flowers, a change which is due to a very active oxidase. It would be of interest to determine the relation if any between the activity of this oxidase and the percentage of essential oil in order if possible to control the flavour and odour of products made from the mahua.

Deodorisation of Mahua Spirit.

In the Pharmaceutical Journal Vol. 31, July 30, 1910, p. 141, a process is quoted for deodorising mahua spirit by digesting with solid potash and redistilling. Experiments by Mr. Mutyala showed that by standing over a mixture of lime and charcoal the odour was greatly improved. Some special treatment of mahua spirit in this way is necessary before using it as a solvent for perfumes or for any purpose where the odour is of importance.

Mahua Syrup.

If its somewhat unpleasant flavour could be eliminated or improved the syrup extracted from mahua flowers would make an excellent substitute for honey or treacle and might be used as a source of sugar for fruit preserving.

Experiments made by Mr. Habib Hassan in this direction met with a considerable measure of success. By boiling the extract of the flowers with lime, filtering and removing the excess of lime with carbon-dioxide, and evaporating the liquid to a syrup in vacuo, a very palatable product was obtained, from which jam and sweetmeats could be prepared. The colour of the syrup was improved by treatment with sulphur dioxide or animal charcoal.

There is little doubt that if the flowers could be utilised for the preparation of syrup immediately on falling from the tree, before they have time to darken or before the cane-sugar percentage begins to decrease, a very satisfactory syrup of the nature of honey could be prepared and would be a valuable source of edible sugar.

VI. Summary and Conclusions.

Microscopical, chemical and fermentation studies have been made to determine the origin and character of the sugars in the mahua flower.

The flowers were examined in four stages:—

1. Bud completely closed.
2. Bud closed but style protruding $\frac{1}{4}''-\frac{1}{2}''$.
3. Flower partly open, style protruding about 2".
4. Flower ripe and ready to shed corolla.

Maltose and glucose were identified microscopically by means of their osazones in sections of the ripe corolla.

Starch was found to be present in the stem and in the pedicle but not in the corolla. Glucose was also found in the stem by ordinary chemical methods.

Tannins were found in abundance in all sections.

An examination of the latex did not reveal the presence either of inulin, starch or sugar.

By careful chemical examination of the flowers at different stages the following carbohydrates were identified, dextrose, levulose, maltose, cane-sugar, pentoses, cellulose. The results of analyses at the different stages show that the total sugar goes on increasing as the flowers develope and is greatest when the flowers are ready to drop.

In the growing stages levulose is always present in greater amount than dextrose. In the final stage the quantities approximate but do not become equal.

The percentage of cane-sugar increases up to the point of the shedding of the corolla. After this and during storage the percentage of cane-sugar decreases relatively to invert sugar.

The total sugar content appears to vary in different varieties of the tree, a small variety of *Bassia longifolia* yielding as much as 80% while the more general percentage is from 60—70%.

A systematic study of the enzymes present in the various stages in the growth of the flower was made. The following were definitely identified.

Amylase, catalase, emulsin, invertase, maltase, oxidase.

The amylase was found to disappear as soon as the flower opens. The same is the case with emulsin. Invertase decreases about the fourth stage. Maltase, catalase and oxidase are present throughout.

The detection of emulsin indicates the presence of glucosides during the first and second stages of the development of the flower and that they disappear after the flower opens.

An examination of the *enzymes in the latex* shows the presence of oxidase and peroxidase but no catalase.

There appears to be a greater percentage of *essential oil* in the large flower of the *Bassia longifolia* than in the smaller variety.

Experiments have been carried out with a view to obtaining the best possible yield of alcohol from mahua flowers.

Three varieties of yeast were found to occur naturally in the mahua flower, these were separated and used for pure culture fermentations free from moulds and acetic and other bacteria. By the use of pure cultures inoculated into a sterile mash of mahua flowers and the addition of such reagents as sulphuric acid and ammonium phosphate yields of alcohol up to 90% of the theoretical have been obtained.

The study of the sugars present in the flowers and adjacent parts and the accompanying enzymes would lead to the conclusion that the parent substance for the sugar in the flowers is the starch in the stem. It was thought not unlikely that the starch elaborated by the leaves in proximity to the flowers, might serve directly to feed the flowers but this cannot be the case because in some trees under investigation the flowers had reached practically the third stage, while the leaves were in an exceedingly rudimentary condition and very small in size, particularly in the case of *Bassia longifolia* proper.

The practical value of these studies is seen in the possibility of obtaining a greater percentage both of total sugar and especially of cane-sugar by increased care in the collection of the flowers and their storage. By extracting the flowers with hot water immediately after dropping a very satisfactory syrup should be obtained and the belief at one time held that mahua flowers would be a valuable source of crystallisable sugar may be to a certain extent realised.

It is possible that pressing of the flowers in situ in compact masses might, by excluding air, result in arresting change.

It would seem possible by careful selection and attention to the conditions of growth to obtain a greater average yield per tree than is at present generally met with.

The season when the fresh flowers can be scientifically studied is a very short one and as it appears impossible to send them long distances without rapid changes occurring in transit, it is hoped that other workers living in the vicinity of mahua trees will repeat and either confirm or extend these inquiries.

Besides the further investigation of the bio-chemistry of the flowers, especially e. g. the conditions of activity of the oxidase present, and the more exact control of the alcoholic fermentation of the saccharine extract, the satisfactory and economic disposal of the effluent from the fermentation process and of the exhausted solid residue from the flowers are problems awaiting solution.

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