CONTRIBUTIONS TO THE STUDY OF THE PRICKLY PEAR PROBLEM

By Gilbert J. Fowler and B. Gopalakrishnamurti.

The studies described in the following paper were undertaken with the object of finding, if possible, some method of utilising prickly pear which would diminish the cost of eradication.

The whole subject is very fully dealt with in the *Report for the Prickly Pear Travelling Commission*, published in Brisbane by the Government of Queensland, Australia, in 1914. The Commissioners, Messrs. T. Harvey Johnston and Henry Tryon visited most countries of the world where prickly pear grows freely, including India. Their attention was mainly directed to the problem of destroying prickly pear by natural enemies, such as rodents, insects and disease, or its 'destruction by utilisation.' Among the chief methods proposed are utilisation as (*a*) a source of alcohol; (*b*) a soil fertiliser; (*c*) a fodder for stock. Among minor possibilities suggested are utilisation of the mucilage, the fibre, and the colouring matter of the fruit, or the production of oxalic acid from the whole plant.

The following investigations are concerned chiefly with the possible uses of prickly pear as a source of alcohol and as a fertiliser. The experimental work falls under three heads :—

1. The microscopical and chemical examination of prickly pear, both of the wild and spineless varieties.

2. The fermentation of prickly pear by various organisms under different conditions.

3. The study of the changes taking place when prickly pear is mixed with soil, especially in reference to nitrogen fixation.

The greater part of the work was carried out with wild prickly pear growing near the Institute, corresponding in appearance with the *Opuntia dillinii*, described in the Report of the Prickly Pear Commission.

For some years, however, one of us (G.J.F.) had been attempting to grow a variety of spineless cactus obtained from the Agricultural Department at Lucknow, in order to see whether it could be cultivated

in dry, barren soil like the spiny varieties, and so afford fodder for cattle in times of drought. No great success was obtained until a certain amount, though small, of water and manure was given to it. The opportunity was therefore taken, in connection with the present research, of examining further the conditions of growth and the composition of spineless cactus. In addition to the Lucknow plant, four other varieties, not very strictly defined, were obtained through the courtesy of Mr. Huidekoper, Head of the Department of Agriculture of the National College, Madras, and were planted on December 8, 1921.

1. MICROSCOPICAL AND CHEMICAL EXAMINATION

Microscopical. Sections were cut with a free razor and examined microscopically after addition of suitable reagents. Traces of starch were found along the edges of the section while the section was fresh; on keeping, however, these disappeared. No reaction for reducing sugars was given by phenylhydrazine, in the absence of preliminary hydrolysis. Normal or hemi-celluloses were not recognised, but characteristic tests for lignin were obtained. No reaction for tannins was obtained. This suggests a possible reason for the failure of the lac-insect to grow on prickly pear, as nearly all the host plants of lac contain tannins in appreciable amount. The bulk of the section consisted of mucilage, which could be easily recognised by its swelling in water and its avidity for such stains as Bismarck Brown, Picric Blue, Light Green, etc. Star-shaped crystals of calcium oxalate were found all over the section. They increased in number on adding calcium chloride, and were soluble in hydrochloric acid, indicating the presence of free oxalic acid as well as calcium oxalate. The sections, it may be noted, were always acid to litmus.

Examination of the milky sap (latex) of the unripe fruit revealed the presence of considerable quantities of calcium oxalate, but no starch or reducing sugars. On boiling with hydrochloric acid and resorcinol, a red coloration appeared, indicating the possible presence of inulin.

Chemical. The analysis of the ash, the determination of crude fibre and of nitrogen, were made by the customary methods given in the handbook of the Association of Official Agricultural Chemists (A.O.A.C.). We are indebted to Mr. Rege for help in this portion of the work, particularly the determination of potash and phosphates, and repeated estimations of ash. The results of these analyses are given in the Table, figures in all cases being the mean of two determinations. Mr. Krishnamurti's analyses were all made in dry

weather, and Mr. Rege's after rain. In the case of the spineless cactus, the amount of manure supplied was only the usual quantity initially employed in making the bed for the reception of the plants, and during dry weather these were watered about twice a week. The photographs given as a frontispiece to the paper show the appearance of the spineless cactus on October 21, 1922, ten months after planting and again on July 14, 1923, a further period of nine months. It can be seen that even with the minimum of attention just mentioned the rate of growth has been considerable.[1]

It is evident from the analyses that the composition of prickly pear is by no means constant, and will doubtless vary between the dry and the rainy season. On the whole, however, it may be concluded that the cultivated 'spineless' varieties of prickly pear contain a greater proportion of water-soluble material and of nitrogen than the wild spiny species. This result agrees generally with the analyses given by J. C. Brunnich of the Department of Agriculture, Brisbane on p. 60 of the Commissioners' Report, which are here quoted for convenience of comparison.[2]

Variety.	Air dry plant.	
	Water-soluble	Total Nitrogen
Spiny Pest Pear Gayndah	39·50	0·774
Dulacca Pest Pear (*O. inermis*)	52·40	0·745
Giant Red Mexican (Westwood Pear) ...	49·80	0·858
Emerald Tree Pear (*Nopalea cochinelifera*)	35·65	0·842
Helidon Tree Pear (*O. tomentosa*) ...	42·46	0·829
Giant Mexican, spineless var. ...	41· 9	1· 41

It may be noted that although the proportion of crude fibre is fairly high, the actual fibres are very short and brittle, and in the opinion of Dr. Marsden, Industrial Chemist to the Government of Madras, have little value for paper making.

For the study of possible fermentations, identification of the sugars and other carbohydrate material present in prickly pear is of first importance. As stated above, the greater part of the plant consists of mucilage. This is difficult to filter and to free from intermixed tissue, fibre, etc. Incipient fermentation of the crushed prickly pear facilitates the separation of the mucilage, but there is danger of more or less complete decomposition (see p. 179). It was found best to boil the minced prickly pear with water and to filter through muslin. The viscous solution thus obtained could not be readily clarified by filtration through paper or by coagulants, such as basic acetate of lead

[1] Cf. Note on 'Spineless Opuntia in Southern India' *Agric. J. India*, 1923, 18, 417.

[2] Cf. also Horn and Mutkekar *Agric. J. India*, 1914, 190.

or alumina cream. On adding 90 per cent. alcohol, the mucilage was precipitated, and did not reduce Fehling's solution. It could be air-dried, the dried material swelling again on addition of water. A few experiments on its possible use as an adhesive both alone and mixed with glue did not give very encouraging results.

According to the analyses quoted in the Prickly Pear Commission Report the mucilage appears to consist largely of galactan. In order to break down a gum of this nature prolonged hydrolysis is necessary if dilute acid at low temperature is employed. It was found more satisfactory to use sulphuric acid of 2–6 per cent. strength at a pressure of 5–10 lbs. Either the mucilage or the whole plant was subjected to this treatment. Galactose was detected in the mucilage after hydrolysis by preparing mucic acid, according to the method given by Onslow[1] which consists briefly in concentrating the solution, making slightly alkaline with caustic soda, oxidising with nitric acid, evaporating and cooling. A white micro-crystalline precipitate of mucic acid was obtained, agreeing in crystalline form and melting point with mucic acid prepared from galactose.

To investigate the products of hydrolysis of the whole prickly pear, the acid solution was filtered from the original pulp of cuticle, spines, etc., neutralised with barium carbonate, filtered and evaporated. We are indebted to Dr. Marsden for a careful investigation of the saccharine syrup thus obtained, and the following description of the methods employed are taken from his notes.

The syrup was taken up with 90 per cent. alcohol, and the extract again evaporated and taken up with alcohol two or three times. The syrup was sweet tasting but did not crystallise. The aqueous solution with phenylhydrazine and sodium acetate gave a mixture of osazones, but an attempt to separate them by fractional crystallisation was not satisfactory; products of well-defined crystalline form were obtained, appearing in some cases to be homogeneous, but the melting points did not coincide with those of known osazones, and no definite conclusions could be drawn. It was therefore decided to attempt a separation by the use of substituted phenylhydrazines, according to the method of Votoczek and Vondraczek.[2]

At the outset, it was observed that addition of a small quantity of ether to the alcohol solution of sugar produced a milkiness, which sank to a slightly yellow, viscous syrup. Ether was therefore added until there was apparently no further separation, and the deposit was heated as before with 8 per cent. sulphuric acid and the solution again worked up for sugars in the manner already described.

[1] *Plant Biochemistry*, pp. 46 and 67, cf. also *Analyst*, 1917, 42, p. 23.
[2] Ber., 1904, 37, 3854

The solution from which the syrup had been precipitated by ether, and that of the rehydrolysed syrup, were treated separately as follows :—

(*a*) The solution in alcohol from which syrup had been precipitated by ether, was evaporated to dryness under diminished pressure, dissolved in water, and 5 c.c. of the aqueous solution (about 0·5 gm. sugar) were treated in a wide test-tube with 0·6 grams sodium acetate crystals and 0·5 grams diphenylhydrazine hydrochloride, with sufficient alcohol to keep the latter in solution. The mixture was gently heated on the waterbath (75–80°) for about one hour. There was no deposit on cooling, but on adding a little ether and leaving to stand, a dark, oily layer collected on the surface and gradually deposited a precipitate. This was filtered, washed with a little ether and recrystallised from alcohol, forming clusters of long needles melting at 187°, and resembling arabinose diphenylhydrazone which melts at 198° ; glucose, mannose and galactose diphenylhydrazones have much lower melting points and the sugar therefore appears to be arabinose. The filtrate from this was concentrated somewhat and, after filtering, was treated with 0·25 grams methylphenylhydrazine and acetic acid. Upon standing the colour became yellowish, but there was no deposit even on standing overnight. Galactose was apparently absent from this portion. The solution was therefore treated with phenylhydrazine and acetic acid in the usual manner for the production of osazones. A voluminous precipitate was obtained after half an hour, and after one hour's heating was washed with hot water and recrystallised from alcohol. The first crystals obtained were definitely characterised as glucosazone, by shape and melting point (204°). Glucose is therefore present in relatively large quantity as a product of hydrolysis of prickly pear mucilage.

(*b*) The syrup precipitated by ether and again heated with 8 per cent. sulphuric and under pressure, was examined in exactly the same way. Upon warming at 80°, with diphenylhydrazine, cooling and covering with ether, there was a precipitation of arabinose diphenylhydrazone which, crystallised from alcohol, melted at 194°. The original filtrate was filtered again until clear and then treated with methylphenylhydrazine and acetic acid ; a deposition began in about ¾ hour, and after 5 hours, when the precipitate began to turn yellow it was filtered off and recrystallised, melting at 184° and, after recrystallisation from water, at 187° (*d*-Galactose methylphenylhydrazone melts at 188–191°). Bromophenylhydrazine and phenylhydrazine were added successively to the filtrate, and the precipitated compounds were recrystallised from alcohol, but the melting points and appearance did not agree with those of definite bromophenylhydrazones or phenylosazones and probably represented mixed derivatives. The

sugars thus characterised as present in the hydrolysed material, therefore, are arabinose, galactose and glucose, the presence of galactose having already been detected by the production of mucic acid.

The total reducing sugars present after hydrolysis as determined by titration with Fehling solution were found to vary within wide limits, according to season, etc., from 3·5–10·5 per cent. The pentoses in the prickly pear were determined in the usual way by distilling with hydrochloric acid and estimating the furfurol in the distillate as a hydrazone. The results of the examination indicated that pentoses were present to the extent of 0·8–1·0 per cent. The total oxalic acid was obtained as calcium oxalate by the method described by Gregoire and Carpiaux[1] the precipitated calcium oxalate being collected and titrated against standard permanganate.

The general approximate composition of the prickly pear used in the investigation may be summarised as follows :—

Moisture ...	... 85·00 per cent.	Crude fibre ...	... 2·15 per cent.
Total dry matter	... 15·00 ,,	Pentoses ...	... 0·90 ,,
Ash ...	... 1·82 ,,	Total Oxalic acid	... 0·55 ,,
Water-soluble portion	... 3·48 ,,	Total reducing sugars after hydrolysis	... 3·5–10·5 ,,
Acidity, water-soluble portion ...	... 0·13 ,,		
Nitrogen ...	... 0·14 ,,		

2. FERMENTATION OF PRICKLY PEAR.

(*a*) *Alcoholic.*—As already stated, the best yield of reducing sugar was obtained by hydrolysing under 5–10 lbs. pressure with 6 per cent. acid. The solution thus obtained, however, after neutralisation with calcium carbonate, concentration and sterilisation, gave little or no fermentation with a pure culture of distillery yeast. The results of the chemical examination already described indicate that glucose is only present as one among other more difficultly fermentable sugars. It may also be that the general composition of the solution obtained on hydrolysis is toxic to the normal alcohol fermentation. In any event, the optimum percentage of sugars fermentable to alcohol by yeast can be only small. Attempts to ferment the fruit of the prickly pear were also not very successful, the juice being very thick and difficult to clarify.[2]

[1] *Analyst*, 1912, **37**, 564.

[2] Mr. Gopalakrishnamurti, who is now Bacteriological Assistant at the Government Distillery at Nasik, has made some further investigations of prickly pear fruit, growing in that district and writes, ' The fruit of a species of prickly pear examined at Nasik, however, contained fermentable sugar to the extent of 8 per cent. practically all of which was found to exist as a monosaccharide, showing no increased cupric reducing power on hydrolysis. It was successfully fermented by the yeast in the mahua flower and also by a yeast present in the prickly pear itself and in each case almost theoretical yields of alcohol were obtained. It contained less mucilage than the Bangalore variety and could therefore be fermented with greater ease.'

(*b*) *Acid.*—It has already been mentioned that if pieces of prickly pear are left in contact with water, fermentation sets in with evolution of gas. That the fermentation is derived from bacteria present in the prickly pear is indicated by the following experiment. Prickly pear was cut into pieces under aseptic conditions and equal quantities put into two sterile bottles. The contents of one bottle were simply washed with sterile distilled water and allowed to ferment practically in absence of air, the bottle being filled up with sterile water, and arrangements being made for collecting the gases evolved. The contents of the second bottle were first washed with 5 per cent. copper sulphate solution and then with sterile distilled water, and the bottle finally filled up with sterile distilled water, as in the case of the first bottle. Both were kept at room temperature. After a day or two gas was evolved from the first bottle, but none from the second. The gas evolved from the fermenting prickly pear consisted chiefly of hydrogen and carbon dioxide with traces of methane. Traces of butyric acid were indicated by odour.

The fermentation of prickly pear in presence of a small quantity of septic tank sludge resulted only in more rapid decomposition, the gaseous products of fermentation being the same, with a strong smell of butyric acid. The mucilage completely disappeared, the final product being a clear, mobile solution, containing only floating spines and fibre. Gas analyses concurrent with fermentation showed that the percentage of carbon dioxide steadily increased at the expense of the hydrogen. Apart from the effect of the gradual saturation of the solution with carbon dioxide, it would appear possible that the increased proportion of this gas may be partly due to the fermentation of calcium oxalate, and to secondary fermentation of the acids produced by the main fermentation of the mucilage. Prickly pear also rapidly fermented when aerated in contact with a little septic tank sludge, the solution increasing in acidity for some time and then becoming neutral.

The possible complete disruption of prickly pear under both ærobic and anærobic conditions is of interest in connection with its use as a green manure.

(*c*) *Attempted fermentation of Prickly Pear to Acetone.*—It was thought possible that prickly pear mucilage might prove a suitable pabulum for the acetone bacillus, in which case valuable products could be obtained by direct fermentation apart from preliminary hydrolysis. A sterile mash of prickly pear was therefore prepared by heating the crushed substance with water for four to five hours under 15 lbs. pressure. It was inoculated after cooling with a pure vigorous culture of the acetone bacillus. Fermentation began within

twenty-four hours accompanied by considerable frothing. Inflammable gas was evolved and the characteristic smell of the acetone-butyl alcohol fermentation obtained. A quantitative fermentation with 200 grams of prickly pear gave only 1·2 per cent. of acetone calculated on the steam-dried material as determined by Messinger's iodoform method.

It would appear that the yield of acetone, such as it is, is really derived from the small percentage of starch originally present in the prickly pear, as the mucilage, *per se*, could not be fermented. Apart from the small yield prickly pear is a difficult material to deal with in a fermentation process, owing to the long period necessary for the completion of the fermentation, and also the frothing which takes place during fermentation and the subsequent distillation. Attempts to minimise the frothing by previously drying, powdering and autoclaving the material resulted in practically no fermentation taking place. The cause of this is difficult to understand, unless it be that the oxalic acid present exercised greater effect on the organisms in a less mucilaginous medium.

An attempt was made to ferment prickly pear, together with 'jawari,' having first established a satisfactory fermentation with the latter substance by itself. The yield of acetone, however, did not materially exceed the amount due to the jawari alone, while the fermentation was somewhat slow in beginning.

3. PRICKLY PEAR AS A GREEN MANURE.

The comparatively small percentage of nitrogen present in prickly pear would lead to the conclusion that the benefit which is evidently obtained from its use as a green manure is due to its assisting the fixation of nitrogen. Accordingly experiments, in some of which we were assisted by Mr. Rege, were made to determine whether the carbohydrate material of prickly pear could supply energy to the nitrogen-fixing organism. The experiments were carried out on similar lines to those of Hutchinson, in his investigation of the nitrogen-fixing power of plant residues.[1]

Two grams of steam-dried prickly pear (finely powdered), 1 gram calcium carbonate, 0·02 gram potassium hydrogen phosphate and 10 grams of sand were added to each of four Erlenmeyer flasks of 250 c.c. capacity and sterilised. Then 10 grams of soil in which prickly pear had been buried some four years (obtained from the Kolar district by the courtesy of Mr. Ramanathan) were added to each of

[1] *J. Agric. Sc.*, 1918, 9, 92-111.

the four flasks and also 30 c.c. of sterile water, enough thoroughly to moisten the contents. One of the flasks was again sterilised and kept as control, along with the others at room temperature. In two of the flasks, the brown film turning gradually to black, characteristic of *azotobacter*, appeared in course of time. In the third flask some moulds appeared. The control flask showed no sign of growth.

The total nitrogen in all the flasks was estimated after twenty-five days by the Kjeldahl method, as follows :—

Total Nitrogen	Control flask.	Flask I.	Flask II.	Flask III.
in mgms. ...	14·23	26·29	23·71	17·02
Gain ...	...	12·06	9·4	2·79

In flasks I and II there is distinct gain in the nitrogen content. The low result in flask III may be due to the presence of moulds. In all these cases it was evident that not much butyric acid was produced, only the faintest smell of it was perceptible on concentrating the contents of the flasks with dilute sulphuric acid prior to the Kjeldahl process, in fact a pleasant odour was noticed during the concentration of the contents of flasks I and II. These observations are of interest in comparison with those made in the next experiment.

In this an attempt was made to determine the nitrogen-fixing power of raw prickly pear on a slightly larger scale. A hundred grams of raw prickly pear pieces, 100 grams sand, 5 grams calcium carbonate, 1 gram potassium hydrogen phosphate and 50 c.c. of water were added to each of two wide-mouthed bottles, of 1½ litres capacity and sterilised, the bottles being closed with rubber stoppers, containing one hole plugged with cotton wool. After sterilising, 100 grams of soil in which peas had been grown were added to each bottle; one was further sterilised to serve as control, and both were kept at laboratory temperature for 31 days. In the control bottle no signs of growth could be observed; in the other, some moulds appeared at the beginning and a white scum formed on the surface of the contents. Gradually, however the moulds disappeared, and the whole film turned brown and finally black. The prickly pear, except the fibre and spines all went into solution. On opening the two bottles after the period of incubation, the control bottle was perfectly sound, whereas the experimental bottle smelt of farmyard manure. During the preliminary evaporation with dilute sulphuric acid large quantities of butyric acid as judged by the smell, escaped from the experimental bottle. The contents of the control bottle emitted no offensive odour. 10 grams of the residue after evaporation with dilute sulphuric

acid were submitted to the Kjeldahl process, and the nitrogen in the total contents of the flasks calculated with the following result:—

Total nitrogen	Mgms.
Experimental bottle	134·5
Control bottle	123·4
Gain in nitrogen	11·1

The gain is distinct though small, and the diminished amount as compared with the first experiment may be due to the increased amount of butyric acid formed. It has been shown that butyric acid is toxic to the nitrogen-fixing organism [1] and unless rapidly neutralised by the calcium carbonate it might exert a prejudicial effect. In the first experiment 1 gram of calcium carbonate was present to 2 grams of steam-dried prickly pear, in the second 5 grams calcium carbonate to about 16 grams dried prickly pear. A second similar experiment gave rather better results but still not as high as the first, viz., a gain of 28·6 mgms. nitrogen per 100 grams raw prickly pear and per 100 grams raw prickly pear after drying, 20·44.

It is true that the soils used for inoculant in the two cases were different, but *azotobacter* was found in both. The low result in the two later experiments would seem to show the wisdom of the ryot's custom of burying prickly pear for a long time prior to its actual use. It may be assumed that the butyric acid fermentation is thus allowed to complete itself and the anærobic gases, hydrogen and carbon dioxide to disappear. If adequate carbonate of lime is present, the calcium butyrate formed may be further fermented and its final oxidation to carbonate of lime furnish energy for nitrogen fixation. This has been shown to be possible by the experiments of Miss Mockeridge.[2]

CONCLUSIONS.

The investigation described in the foregoing pages leads to the following conclusions:—

1. That sufficient material fermentable by yeast is not present in prickly pear, nor can be produced from it by acid hydrolysis to make it of any value as a source of power alcohol. The fruit, on the other hand, contains fermentable sugar but successful fermentation of the fruit will largely depend on its actual sugar content and the

[1] *J. Agric. Sc.*, 1907-8, 2, 35-51.
[2] *Biochem. J.*, 1915, 9, 272.

cheapness with which it can be collected and transported to the distillery. At any rate it is very unlikely that it will compete with other cheap raw materials as a source of alcohol.

2. That while prickly pear readily ferments spontaneously both under ærobic and anærobic conditions yielding butyric acid, hydrogen and marsh gas, no success has attended attempts to produce acetone from it by a pure fermentation with the acetone bacillus.

3. That prickly pear is a valuable source of energy for the nitrogen-fixing organism, provided sufficient carbonate of lime is present to neutralise all acid formed.

4. That several varieties of ' spineless ' cactus can be readily grown in Southern India with a very small provision of water or manure, and they contain a higher percentage of water-soluble material and of nitrogen than the wild spiny species.

It is a pleasure to us to acknowledge with many thanks our indebtedness to Dr. Marsden for undertaking the complex investigation of the mixture of sugars obtained on hydrolysis, to Mr. Rege for completing the analyses and experiments referred to in the text, after Mr. Gopalakrishnamurti had left the Institute to take up his appointment at Nasik, and to Mr. Banerjee for taking the photographs shown in the frontispiece.

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TABLE

Name of the plant	Steam-dried plant							Green plant						
	Total dry matter	Ash	Water soluble portion	Crude fibre	Nitrogen	Phosphate P_2O_5	Potash K_2O	Moisture	Ash	Water soluble portion	Crude fibre	Nitrogen	Phosphate P_2O_5	Potash K_2O
Spiny variety (prickly pear) ...	100	12·1 23·64 R	23·2	14·35	0·96	·1015	1·499	85	1·82 3·55 R	3·48	2·15	·14	·015	·225
Spineless cactus—														
Sugar variety (Adyar) ...	100	29·86 24·85 R	53·2	11·5	1·45	·1259	1·46	91	2·67 2·24 R	4·75	1·02	·13	·011	·131
Local variety (Lucknow)...	100	26·10 19·39 R	45·5	14·6	0·94	·0917	7·79	91·4	2·24 1·67 R	3·91	1·26	·08	·008	·067
Special species ...	100	25·50 25·15 R	...	14·49	1·17	·1406	1·445	90·3	2·47 2·44 R	...	1·41	·11	·014	·140
A spineless cactus ...	100	20·61	...	...	1·45	...	...	...	...	...	...	...	...	...

N.B.—Mr. Rege's figures for ash are marked by R.

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