https://doi.org/10.29321/MAJ.10.A04810

and some of them such as *Acherontia, Herse, Hippotion, Theretra* and *Deilephila* are plant pests of some importance. As adult moths most of the sphingids are either harmless or helpful. The harm done has been noted chiefly with the death's head moth *Acherontia styx*, which has been often reported to rob honey from bee hives. The writer doubts whether this moth causes any appreciable loss in honey in the case of artificial hives where it is impossible for it with its stout body to enter a hive through the small bee hole. As regards beneficial work done it may be added that several species of hawk moths, especially the crepescular and diurnal species like *Macroglossa, Cephanodes* etc. carry on good work as pollinators; their long tongue helps them in reaching some of the deep flowers like those of the Convolvulaceae.

It is quite possible that further studies might add a good deal to our present knowledge of Indian hawk moths and their economic importance.

References to Literature.

Lefroy, M. — Indian Insect Life, 1909

Fletcher, T. B. — Some South Indian Insects 1914.

Ayyar, T. V. R. — Life history description of *Cardia sphinx* (*Polyptychus*)—Rpt. of 3rd Ent. Meeting Pusa, 1919, (with plate).

— — Notes on insect parasites from S. India, *Journal of Bombay, N. H. Socy.*, Jan. 1925, (with figures).

— — Bulletin on Insect pests of crops in S. India. *Bull. No. 27, Madras Dept.*, 1932.

— & Margabandhu, V. — Hymenopterous parasites of economic importance in S. India. *Madras Agri. Journal*, Dec. 1934.

THE CHEMICAL COMPOSITION AND ENZYME CONTENT OF INDIAN HONEY

By K. VENKATA GIRI, M. Sc., A. I. I. Sc.,

(Department of Biochemistry, Indian Institute of Science, Bangalore).

Figures for the composition of honey commonly used in other countries are known, but there is no information available regarding the composition of Indian honeys. The present investigation was, therefore, undertaken as a preliminary step towards the analysis of Indian honey samples, for the purpose of purity determinations in order to set standards by which the purity of honey could readily be ascertained.

The study of the analysis of the honey samples has now been made by the most reliable methods which can be obtained. Sucrose, levulose and glucose have each been determined separately. Since these are the only carbohydrates which are absorbed as sugars from the gut, their sum constitutes the available carbohydrates of honey.

Materials and Methods. All the honey samples were obtained through the courtesy of Messrs. The Coorg Honey and Wax Producers' Co-operative Society, Ltd. They were collected from three different places and were known therefore to have been collected by bees under different floral conditions

The methods used for the preparation of the sample for analysis and for the determination of water and ash were those described in A. O. A. C. Reducing sugars were determined by Lane and Eynon's (1923) copper titration method. Total sugar was estimated after inversion with hydrochloric acid. The difference between the percentage of total sugar and of reducing sugar, calculated from Lane and Eynon's invert sugar table, gave the percentage of sucrose in the honey.

The iodometric procedure described by Lothrop and Holmes (1931) was adopted for the determination of fructose/glucose ratio. The values for fructose and glucose were calculated by solving the simultaneous equation obtained from the iodometric determination and from the estimation of the reducing power by Lane and Eynons copper method.

Diastatic number. The diastatic number of honey was determined according to the method of Gothe (1914).

Phosphatase activity. The presence of the enzyme phosphatase in oney was shown for the first time. The activity of the enzyme was determined as follows :—

10 c. c. of honey was diluted to 20 c. c. with water. 5 c. c. of the honey solution was added to a reaction mixture containing 10 c. c. of N/5 acetic acid-acetate buffer of pH 5·2, and 5 c. c. of 2 per cent. sodium-β-glycerophosphate, with or without magnesium chloride, the total volume of the mixture being adjusted to 25 c. c. The reaction mixture was incubated at $35° \pm 0{\cdot}1$ for 24 hours. At the end of the period 10 c. c. of the mixture were removed and added to 10 c. c. of 10 per cent. trichloracetic acid, filtered and the inorganic phosphorus was determined by the method of Fiske and Subbarow (1925). The activity of the phosphatase is expressed in terms of mg. P liberated in the total volume (25 c. c.) of the reaction mixture after 24 hours hydrolysis by 5 c. c. of diluted honey (1 : 1).

The results of the analysis are presented in Table I.

Discussion. It will be noticed that all the honey samples examined contain approximately the same amounts of free levulose and glucose. The sucrose content, however, varies from 0·380 to 1·04 per cent. The results show that the ratios of dextrose to levulose are practically the same for all honey samples investigated (1·1 to 1·18).

It has been suggested that the colour of honey is usually associated with the mineral ash content. There is very little data available on this point in literature. Schuette and Remy (1932) examined a number of samples of honey and have shown that the darker the colour, the higher the proportion of mineral constituents. Further, the dark honeys were found to contain more copper and manganese than the lighter coloured products. The results of the analysis of Indian honeys also support the hypothesis that there exists in honey a relationship between the degree of pigmentation and the mineral content. Thus, honey No 2 (Table 1) which is dark in colour has a higher

Table I.

Number.	Details of samples.	Water %	Ash %	Glucose %	Laevulose %	Ratio Laevulose / Glucose	Sucrose %	Free acid (c. c. of 0·1 NaOH required to neutralise 100 g. of the sample.	Diastatic No.	Phosphatase activity in mg. P in the total volume of R-M after 24 hrs. hydrolysis	
										without mg.	with 0·01 M. mg.
1.	North Coorg honey. Crystallised yellow colour. ...	17·5	0·031	39·2	39·5	1·01	0·380	18	7·1	0·208	0·293
2.	South Coorg honey. Bitter honey Dark colour. (Slightly fermented). ...	22·14	0·46	36·7	36·8	1·00	0·570	64	10·0	0·680	0·780
3.	West Coorg honey. Yellow colour. ...	20·7	0·062	35·8	40·1	1·12	1·04	25	8·3	0·233	0·260
4.	West Coorg honey. Yellow colour.	19·5	0·074	36·1	40·5	1·12	1·00	22	10·0	0·300	0·335
5.	West Coorg honey. Crystallised. Small crystals distributed throughout.	16·0	0·103	34·2	38·5	1·12	0·380	17	6·3	0·375	0·450
6.	North Coorg honey. Yellow colour. Slightly fermented. ...	19·2	0·120	35·1	37·7	1·07	0·760	30	6·3	0·380	0·540
7.	West Coorg honey. Yellow colour. Crystallised. Crystals settled at the bottom.	19·0	0·075	35·1	39·1	1·11	0·380	19	5·0	0·312	0·312
8.	North Coorg honey. Yellow colour. Crystallised. The crystals were distributed throughout. ...	16·2	0·048	36·7	41·4	1·12	0·475	18	5·0	0·153	0·175
9.	West Coorg honey. Crystallised. Crystals settled at the bottom.	19·7	0·043	35·1	38·7	1·10	0·285	21	5·0	0·258	0·258
10.	South Coorg honey. (Processed: heated upto 158°F.)	20·4	0·072	34·2	40·4	1·18	0·760	14	2·0	0·233	0·260
11.	South Coorg honey. (Processed: heated upto 158°F). Yellow colour.	20·9	0·116	34·2	38·7	1·13	0·570	13	2·5	0·180	0·280
12.	Blended and processed and heated upto 158°F. ...	18·7	0·055	35·8	40·1	1·12	0·570	13	2·5	0·190	0·210

proportion of ash. In view of the importance of manganese and copper in the diet, it would be useful to determine the mineral content of various samples of Indian honey.

Titratable acidity recorded in the table for honey samples ranges from 13—25 for normal honey samples, and 30 and above for fermented samples of honey. The results, therefore, indicate that there appears to be some relation between the acidity and fermentation. The high figure for titratable acidity obtained in the case of fermented samples are probably due to the formation of organic acids as a result of fermentation.

The subject of enzymes in honey has been studied rather extensively for a number of years ; much of the work having been undertaken in an effort to find means for detecting adulteration (Auzinger, 1910; Axenfeld, 1903; Feelenberg, 1911; Gothe, 1914 ; Langer, 1903; Lenz, 1910 and Utz, 1908). Much work is being done on the evaluation of honey on the basis of diastase content. Braunsodorf (1931) has suggested the value 23·8 as a lower limit for first class diastatic honeys while 17·9 as the lowest permissible limit for unheated honeys. The present results have shown that the temperature which is generally employed for pasteurisation has noticeably affected the diastase content. All the honey samples examined are found to possess very low diastatic activities. It may be due to the high temperature occurring in the places where the honey has been collected. Many North American, particularly Californian honeys have been found to have low diastatic values (Bartels and Fauth, 1931) and it has been suggested that the high temperature occurring in many districts of California accounts for the low enzymic content of the honey. Fiehe (1930) also observed that Californian honeys have a very low diastatic value, and suggested that 8·3 be considered as a suitable lower limit of diastatic power, and he states that it is becoming recognised as such in the trade.

It has been shown for the first time that an enzyme which hydrolyses sodium glycerophosphate into inorganic phosphorus occurs in honey. The activity of the honey phosphatase is affected by variations in hydrogen-ion concentration and the enzyme was found to be active at a pH range of about 4·5 to 6·5. The enyzme was found to be activated by magnesium. The results show that fermented honey samples show characteristically high phosphatase activity, while the values for unfermented samples are decidedly low. The effect of pasteurising honey is to lower the phosphatase activity somewhat. It is suggested that honey phosphatase is derived chiefly from fermentation yeast and bees and partly from the plants.

Spoilage of honey held in storage over long periods of time is a serious problem in honey industry. The spoilage of honey is due to two main factors : (1) contamination by sugar tolerant yeasts, and (2) decomposition of sugar when the honey is kept at 80°F or above for a long time. The freshly extracted honey is always infected with sugar tolerant yeasts, which are capable of setting up fermentation under favourable conditions.

The favourable factors for the growth of yeast are coarse crystals, a high water content and the abundance of pollen grains. Excess of moisture has some relation to the spoilage of honey by fermentation. In table I it can be seen that the fermented honey samples contain decidedly high water content. Further, it has been noted that the size of the crystals and the water content are related to each another. The water content increases when the crystals are coarse and large and settle down at the bottom. On the other hand if the water content is low, the crystals are small and form throughout the liquid phase. The top portion of the crystallised honey contains more moisture than the bottom layer. Fermentation begins at the top and slowly spreads down through the honey. For preventing spoilage it is necessary to control the crystal formation. The crystals should be as small as possible. Their formation as small crystals can be controlled by the addition at temperature of about 75°F of 5 to 10 per cent. of a previously processed finely crystallised honey, followed by thorough agitation and mixing. The product can be filled into the final containers and stored for two days at about 57°F. after which the honey can be stored at any temperature without danger of fermentation.

The fermentation is also prevented by pasteurising honey at 130°F. for 30 minutes. At high temperatures of about 160°F. the flavour and colour are spoiled, particularly when followed by slow cooling. It is recommended to pasteurise all honeys before storage or marketing.

Summary. The sucrose, glucose, laevulose, water, ash, acid, diastatic number and phosphatase activity of genuine samples of honey have been determined. The diastatic numbers of all the samples of honey were found to be too low. For the first time, it is shown that phosphatase occurs in honey.

The author's thanks are due to Prof. V. Subrahmanyan for his keen interest in the present investigation and to the Coorg Honey & Wax Producers' Co-operative Society Ltd., for kindly supplying the honey samples.

References.

1. Auzinger, A. (1910) *Z. Unter. Nahr. Genuss.*, 19, 65, 353
2. Axenfeld, D. (1903) *Zentrabl. physiol*, 17, 268.
3. Bartels and Fauth. (1931) *Z. Unters. Lebensm.*, 66, 396.
4. Braunsdorf. (1931) *Ibid*. 61, 411.
5. Fellenberg, T. von. (1911) *Mitt Lebensmitln Hyg. Eidg. Gendhtsamt. (Switz.)*, 2, 369.
6. Fiehe. (1930) *Z. Unters. Lebensm*, 61, 420.
7. Fiske, C. H., and Subbarow, Y. (1925) *J. Biol. Chem.*, 66, 375.
8. Gothe. (1914) *Zeit. f. Unters. d. nahrgs. u. genussm.*, 28, 273, 286.
9. Lane and Eynon, (1923) J. *Soc. Chem. Ind*, 42, 132T.
10. Langer, J. (1903) *Schweiz. Wohnscher, chem u pharm.*, 41, 17.
11. Lenz, W. (1910) *Apoth Ztg.*, 257, 678.
12. Lothrop, R. E. and Holems, R. L. (1931) *Ind Eng. Chem. Anal. Edn.* 3, 334.
13. Schuette and Remy. (1932) J. *Amer. Chem. Soc*, 54, 2909.
14. Utz (1908) *Ztschr. Offentlal. Chem.*, 14, 21.