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STUDIES IN MAHABALESHWAR HONEY-PART 'III' VITAMIN CONTENTS (ASCORBIC ACID, THIAMINE, RIBOFLAVIN AND NIACIN) AND EFFECT OF STORAGE ON THESE VITAMINS.

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Importance of vitamins in human and animal nutrition is now well recognisd. A very large number of publications appear every year relating to the study of (a) vitamin make-up of all types of foods and (b) the ill effects of lack of vitamins in animals. In the present investigation an attempt has been made to study the vitamin content (ascorbic acid, thiamine, riboflavin and niacin) of Harda, Gela, Jambhul and Pisa varieties of honey, since no such data with Indian honeys are available. Further the samples of honey were stored in the dark at room temperature (28-30)°C and at intervals of 6 months and 1 year they were taken and ascorbic acid, thiamine, riboflavin and niacin contents of above honeys were determined.

Experimental and Results

Estimation of ascorbic acid: Five g of honey was extracted with 6% metaphosphoric acid. The extract was made to 50ml volume and it was used for the estimation of ascorbic acid by titrating against 1 ml of 0.025% 2 : 6 dichlorophenol-indophenol dye according to the method of Birch, Harris and Ray[1] until the red colour of dye in acid pH changed to a original pale yellow colour of the honey extract. Results of ascorbic acid estimation are given in table I and II.

Estimation of thiamine, riboflavin and niacin: It is a known fact that thiamine, riboflavin and niacin occur both free and bound and that all these have to be liberated from the bound form before estimation. For this purpose enzymatic digestion as suggested by Harris and Wang[2] was resorted to; also this procedure enables simultaneous estimation of the 3 vitamins.

(1) *Extraction of free thiamine, riboflavin and niacin*: Ten g of honey was mixed with 50 ml of 1M acetate buffer (pH 4.5) and filtered. The filtrate was used for the estimation of free thiamine, riboflavin and niacin.

(2) *Extraction of total thiamine, riboflavin and niacin*: Ten g of honey was mixed with 25 ml of acetate buffer pH 4.5 (1 M). To this, 50 mg each of papain and Taka diastase and 2-3 ml of toluene were added. The mixture was incubated at 43°C for 18 hours. After completion of enzymic digestion, the mixture was kept in a water-bath at 60°C for an hour, to destroy the enzymes, cooled, filtered and made to 50 ml. To the filtrate 17 g of ammonium sulphate was added to precipitate the proteins. After 30 minutes it was filtered through whatman No 44 filter paper and the filtrate was made to 100 ml and used for the estimation of thiamine, riboflavin and niacin.

Table I. *Ascorbic acid content of honey*

Samples	mg / 100 g of honey
Harda	2.27
Gela	2.40
Jambhul	2.00
Pisa	3.32

Table II *Effect of storage at 28-30°C on ascorbic acid content of honey*

Samples	(mg/100 g of honey) Time of storage in months 0 initial	6	12	% loss in year
Harda	2.27	2.05	1.84	19.00
Gela	2.40	2.24	1.92	19.20
Jambhul	2.00	1.80	1.70	15.00
Pisa	3.32	3.06	2.60	22.70

Estimation of thiamine: Five ml of the filtrate was shaken with 3 ml of alkaline ferricyanide solution and 10 ml of iso-butyl alcohol to oxidise it to thiochrome according to the method of Harris and Wang[2]. Fluorescence was measured in a Beckman spectrophotometer model DU using fluorescenic accessories.

Estimation of riboflavin: Ten ml of filtrate was treated with 1 ml of 4% $KMnO_4$ and subsequently with 3% H_2O_2 for the removal of interfering non specific fluorescent substances according to the method of Scott *et al*[3]. The fluorescence of this solution was measured in the 'Lumetron' model 402 ef using primary and secondary B_2 filters.

Estimation of niacin : Ten ml of filtrate was pipetted in a conical flask and pH of the filtrate was adjusted to 7.0 by the addition of 2.5 N NaOH, filtered and volume made to 50 ml. This filtrate was used for the estimation of niacin.

To 5 ml of the filtrate 3 ml of CNBr was added. After 20 minutes, 2 ml of 2% aniline was added and the intensity of the colour developed was measured on Beckman spectrophotometer model D U at 540 mμ according to the method of Swaminathan[4].

Results of free and bound vitamins and effect of storage on above vitamins are given in tables III and IV.

Discussion

It can be seen from table I that samples of honey contain 2 to 3.4 mg per 100 g of ascorbic acid. Table II showed that on storage about 20% of ascorbic acid was lost.

The results tabulated in table II showed that samples of honey contain from 8-22 μg of thiamine. Pisa honey contains the largest amount and Gela the lowest. Thiamine in these samples was found to be present entirely in the bound form. The 4 varieties of honey showed 12-54 μg of riboflavin per 100 g. Riboflavin in Harada and Gela honey appears to be present entirely in the bound form, while in Jambhul and Pisa varieties 32% and 18% respectively of riboflavin is present in the free form. Samples of honey showed great variation in niacin content (from 442 μg in Harda to 978μg in Pisa variety of honey). Unlike thiamine and riboflavin,

Table III. *Free and bound thiamine, riboflavin and niacin contents of honey* (μg/100 g of honey)

Samples	Thiamine			Riboflavin			Niacin		
	Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
Harda	—	10.8	10.8	—	16.0	16.0	56.2	385.6	441.8
Gela	—	8.5	8.5	—	12.3	12.3	80.0	662.8	742.8
Jambhul	—	16.0	16.0	14.3	27.2	41.5	91.0	782.6	873.5
Pisa	—	22.4	22.4	10.1	44.0	54.1	106.4	871.8	978.2

Table IV. *Effect of storage at 20-38°C on the total thiamine, riboflavin and niacin content of honey* (μg/100 g of honey)

Sample	Time of storage months									% lost in 1 year		
	Initial			After 6 months			After 12 months					
	Th.	Ri.	Ni	Th.	Ri.	Ni	Th.	Ri.	Ni.	Th.	Ri	Ni.
Harda	10.8	16.0	441.8	9.2	15.2	414	8.6	14.6	406.8	20.5	9.0	8 0
Gela	8.5	12.3	742.8	7.8	11.0	720.2	7.2	9.8	698.5	15.0	20.0	7.3
Jambhul	16.0	41.5	873.5	14.3	37.0	816	13.2	34.6	789	17.5	16.0	9.35
Pisa	22.4	54.1	978.2	20.8	50.2	916.4	19.6	48.0	866 5	12.5	11.3	11.40

major portion of niacin in honey is found to be present in the free form.

From the results given in table IV it can be seen that on storage for 1 year at 28-30°C, about 12 - 20% of thiamine, 9-20% riboflavin and 8% of niacin were lost.

It was observed that the samples of honey retained about 80% of ascorbic acid, 83% thiamine, 88% of riboflavin and 92% of niacin on storage for 1 year at room temperature. These observations compare well with similar observations for the effect of storage with fruit juices. Robert[5] reported that grape fruit juice stored for 9 to 15 months, lost about 25% of its ascorbic acid content. Moschette *et al.*[6] observed that, when canned tomatoes were stored at 85°F for 1 year, the fruit retained 82% ascorbic acid, 82% thiamine, 93% riboflavin and 95% niacin.

Huelin[7] recently reported that anaerobic destruction of ascorbic acid is accelerated by fructose. Fructose is produced in honey by the action of the invertase enzyme on sucrose, when the bees convert nectar into honey. Griebel and Hess[8] reported considerable loss of ascorbic acid, when the bees convert nectar into honey. This explains the presence of small amount of ascorbic acid in honey.

An acid condition has been found to be undoubtedly very favourable for the retention of thiamine, riboflavin and niacin. The higher percentage retentions of these vitamins in honey therefore, are not surprising because of the prevalence of almost optimum levels of hydrogen ion concentration (pH4.5-5) for these vitamins.

From the results it was observed, that of the varieties of honey used, Pisa variety is the richest in all the vitamins under test. This once again supports the statement, that Pisa variety is the most nutritious of the Mahabaleshwar honeys examined.

Summary

(1) Microchemical determination showed the presence in honey of small amount of ascorbic acid and fair amounts of thiamine, riboflavin and niacin.

(2) Thiamine, riboflavin and niacin are found mostly in bound form in honey.

(3) On storage at 28-30°C for 1 year, about 80% ascorbic acid, 83% thiamine, 88% of riboflavin and 92% niacin was retained.

(4) Amongst the varieties of honey tested, Pisa variety of honey was found to be richest in the vitamins studied.

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