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Research Article

QUALITY ASSESSMENT OF SOME INDIAN HONEYS IN STORAGE THROUGH HMF CONTENT AND INVERTASE ACTIVITY

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ABSTRACT

Objective: The present investigation was made for a proper assessment of some Indian honeys through the evaluation of pH, free acidity, moisture content, electrical conductivity and giving special emphasize on Hydroxy Methyl Furfural (HMF) content and invertase activity.

Methods: 21 honey samples from different places of India were collected and physicochemical parameters were studied following the method of International Honey Commission, (IHC) 2009.

Results: In most of the cases (16 out of 21 honey samples) honeys contain higher values of HMF in respect to international standard limit (80mg/kg.) and their invertase activities vary greatly during storage. Product moment correlation between HMF content and invertase activity showed strong negative correlation (-0.6583077) and one-way ANOVA suggests these two parameters are related to the storage duration. The pH values, moisture content, free acidity and electrical conductivity were also measured in accordance to their standard limits.

Conclusion: We can conclude that the high HMF content with lower values of invertase activity in most of the honey samples was perhaps due to their exposure in high heat stress during honey processing and storage, because in tropical countries like India the average normal temperature remains high especially during summer months.

Keywords: Honey, Physicochemical, HMF, Invertase, IHC, ANOVA

INTRODUCTION

In India, honey is used as nutritional supplement, in therapeutics purposes and also in cosmetic products. Therefore, honey has a high commercial significance, although knowledge of the product quality is not adequate. A range of different physicochemical parameters in comparison with their international standard limits may constitute necessary information for the quality control of local as well as apiary honeys available in the market vis-a-vis the acceptability of the products in international market. Among the different physicochemical parameters, hydroxymethyl furfural (HMF) content and invertase activity of honey are the important parameters to evaluate honey freshness and the heating or storage condition effects on honey quality. HMF or 5-hydroxymethyl-2- furfuraldehyde is an aldehyde and a furan compound which is formed after thermal decomposition of sugars and carbohydrates. HMF is found to be present in many food products like honey, fruit juice, syrup, jam etc. [1]. Generally, its presence in honey indicates heat stress, over storage, spoilage, adulteration etc. HMF is not found to be toxic but evidences are there to prove its carcinogenic potential of other members of this class [2]. The HMF plays an intermediate form to produce carcinogenic product but the toxicity of the substance is little known [3]. On the other hand, the amount of invertase activity denotes the freshness of honey. Invertase is commonly responsible for conversion of sucrose to fructose and glucose [4]. It is found to be formed within the hypopharyngial glands of honeybee and then mixed with the nectar to ripen into honey [5]. In commercial processing plants, honey is usually heated to 60°C or above for inhibiting microorganisms, facilitating packing and delaying crystallization [6]. High HMF content indicates deterioration of honey which mainly due to unsuitable conditions during storage and / or heating of honey [7-8]. The most parameters used as indicators of freshness and overheating of honey are HMF, diastase and invertase. HMF and diastase are included as international quality standards for honey, but invertase is considered better than diastase as a freshness index because it is more sensitive to heating [9-12].

A limit of 40 mg/kg has been demarcated by EU for HMF content in honey [13] whereas the limit imposed by International Honey Commission [14] is 80mg/kg. In case of invertase activity there is no standard limit but it is only the parameter for fresh honey. In tropical countries like India the summer temperature remains very high which extends for several months. So, the aim of the present study is to evaluate the amount of HMF along with invertase activity in honey samples which are exposed in high temperature during normal storage period. In this context, an attempt has also been made to properly evaluate and correlate these two major parameters which are directly related with heat stress and honey freshness along with other physicochemical parameters.

MATERIALS AND METHODS

Selection of honey samples

A total of 21 honey samples were collected from 10 different states of India, such as, West Bengal (5 samples), Tripura (2 samples), Orissa (2 samples), Kashmir (3 samples), Himachal Pradesh (2 samples), Uttarakhand (1 sample), Madhya Pradesh (2 samples), Maharashtra (2 samples), Bihar (1 sample) and Karnataka (1 sample) during the year 2009-2012 (Table 1) from the local market, forest office and apiaries. The collected honey samples were stored in room temperature. Different physicochemical parameters of these 21 honey samples were measured. Honey samples were grouped on the basis of storage duration from harvesting time. Four groups were made such as, group 1 (storage <6 months), group 2 (storage >6 months -<1yr), group 3 (storage >1-<2 yrs) and group 4 (storage >2 yrs) (Table 1).

Estimation of pH, free acidity, moisture content and electrical conductivity

Honey samples were analyzed to measure their moisture content, electrical conductivity, free acidity, and pH values (Table 2) following the recommended methods of International Honey Commission, 2009. Moisture contents were measured by calculating the refractive index using the refractometer (NAR-1T, Atago). The pH of the honey samples were measured by digital pH-meter, EC by electrical conductivity meter and free acidity by simple titration method.

Estimation of Hydroxymethyl furfural content

The HMF content of honey samples (Table 2) were determined by White's spectrophtometric method recommended by International Honey Commission, 2009 [14]. It was based on the determination of UV absorbance of HMF at 284 nm. The results were expressed in mg/kg. 5g of honey solution was taken in a 50 ml beaker, dissolve in approximately 25 ml of water, added 0.5 ml of Carrez solution I and

mixed thoroughly. Next, 0.5 ml Carrez solution II was added mixed and made up to 50 ml with distilled water.

Filtered the solution through paper (Watman No.1), rejecting first 10 ml. and then pipetted 5 ml in two test tubes each. In one test tube, 5 ml of water was added (sample solution) and in another 5 ml of sodium bisulphite solution was added (reference solution). The sample solution was measured spectrophotometrically at 284 nm and the reference solution at 336 nm. HMF mg/kg = (A_{284}-A_{336}) ×149.7×5×D/W. D = Dilution factor.

Estimation of invertase activity

Invertase activity of the honey samples (Table 2) were measured spectrophotometrically at 400 nm according to the method of Siegenthaler [15] recommended by International Honey Commission, 2009. The analyses were done by using Shimaddzo UV-1700 UV-visible spectrophotometer. 5g of honey sample mixed with buffer solution and made up to 25 ml. 5 ml of substrate solution was taken and kept in water bath at 40ºC for 5 min, then added by 0.5 ml of honey solution, mixed the contents and incubated at 40ºC. After exactly 20 min 0.5 ml of reaction terminating solution was added and mixed well. For the blank, reaction terminating solution was added before addition of honey solution.

The OD values were taken at 400 nm. Calculation: Invertase in U/kg = 158.94×A_{400}

Statistical analysis

Product moment correlation between HMF content and invertase activity was calculated using R (2.15.0) software. One-way ANOVA for these two parameters were also performed to determine the significance of the groups constructed on the basis of storage duration of the honey.

RESULTS

pH and free acidity

pH of the honey samples varied within 3.12 ± 0.02 - 4.1 ± 0.01 (Table 2) confirming their acidic nature. The amount of free acidity in studied honey samples varied within 27 ± 1.80 - 63.33 ± 10.40 meq/kg. Only two samples H5 and H14 showed higher level of free acidity than the internationally recommended limit (50meq/kg.).

Moisture content

The moisture contents of the studied honey samples showed a quite high amount and range in between 15.86 ± 0.11 - 25 ± 0% (Table 2). Out of the 21 samples, 15samples show values above 20% and five were lower than that. The higher values of moisture content signify probability of fermentation or spoilage of honey samples, probably for premature honey harvesting or by inappropriate storage conditions.

Table 1: List of Honey Samples for Study with Their Storage Groups

S. No.	Honey code No. no.Sample	Place of collection	State	Site of collection	*Group
1.	H1	Bardhaman	West Bengal	Local market	1
2.	H2	Joka	West Bengal	Apiary	2
3.	H3	Moipith	West Bengal	Local market	1
4.	H4	Majhdia	West Bengal	Local market	2
5.	H5	Bishnupur	West Bengal	Local market	1
6.	H6	Bisalgarh	Tripura	Local market	2
7.	H7	Belonia	Tripura	Local market	3
8.	H8	Shrinagar I	Jammu& Kashmir	Apiary	3
9.	H9	Shrinagar II	Jammu& Kashmir	Apiary	2
10.	H10	Solan	Himachal Pradesh	Apiary	2
11.	H11	Sundernagar	Himachal Pradesh	Apiary	4
12.	H12	Pachmari	Madhya Pradesh	Forest office	4
13.	H13	Kanha	Madhya Pradesh	Forest office	3
14.	H14	Shimlipal	Orissa	Local market	4
15.	H15	Keonjhor	Orissa	Forest office	1
16.	H16	Rudraprayag	Uttarakhand	Apiary	2
17.	H17	Kurg	Karnataka	Local market	2
18.	H18	Mujuffarpur	Bihar	Local market	3
19.	H19	Pune	Maharastra	Apiary	1
20.	H20	Aurangabad	Maharastra	Apiary	3
21.	H21	Khrewan	Jammu &Kashmir	Local market	3

*Groups were made based on storage period (e.g group 1= < 6 months, Group 2= 6 months- 1 year, Group 3= 1-2 year, Group 4= > 2 years)

Electrical conductivity

The diverse sources of honey samples (nectar and honey dew) can be ascertained by their varied electrical conductivity values. The origin of the honey samples as blossom or honey dew is based on the value of the EC (Electrical Conductivity) of the honey samples [16-17]. Accordingly, honeys with EC value higher than 0.8mS/cm are considered as honeydew honey, whereas honeys with lower values are blossom honey or blends of blossom with honeydew honey. Generally, honey with EC value lower than 0.5mS/cm is considered as blossom honey and the honey samples with EC values between 0.8mS/cm - 0.5mS/cm are blended in nature [18].

In this present study, EC values vary between 0.15 ± 0.0057 to 1.08 ± 0.0057 (Table 2). So, as per suggested limits, 10 honey samples (H1, H2, H3, H6, H8, H11, H13, H16, H19 and H20) out of the 21 analyzed samples were of blossom honey, eight samples (H5, H7, H9, H10, H15, H17, H18 and H21) were of blended with blossom and honeydew honey and remaining three samples (H4, H12 and H14) were of honeydew honey in origin (Figure 1).

HMF content and invertase activity

HMF content of the honey samples showed a great variation ranging between 47.1 ±0.56 - 642.31 ± 0.69 mg/kg (Table 2, Fig. 2). Most of the samples showed higher values of HMF content than internationally recommended limit (80mg/kg.) which may signify the tropical climatic condition or, poor storage condition or, wrong procedure of processing. Among the 21 honey samples, only five samples (H1, H3, H5, H15 and H19) having the HMF content within the limit range and the remaining 15 samples showed higher values (Fig. 2). Honey sample of Moipith (H3) shows the lowest value of HMF content i.e. 47.1 ± 0.56 mg/kg. with a highest invertase activity of 48.89 ± 1.59 U/kg (Fig. 2). On a contrary, honey sample of Pachmari (H12) shows the highest value of HMF (642.31 ± 0.69 mg/kg.) with a lowest value of invertase activity i.e. 0.476 ± 0.16

U/kg (Fig. 3, Table 2). Invertase activity, in most of the honey samples, is quite low. Bardhaman honey sample (H1) shows HMF

content of 55.68 ± 0.65 mg/kg. which is within limit range and higher invertase activity of 28.23 ± 3.94 U/kg.

The invertase is considered to be the indicator of honey freshness index which is sensitive to heat stress.

In the present work, 15 out of 21 samples showed low invertase activity (Fig. 3, Table 2).

It was also observed in most of the samples that invertase activity of the honey sample is inversely proportionate to the HMF content (Fig. 3).

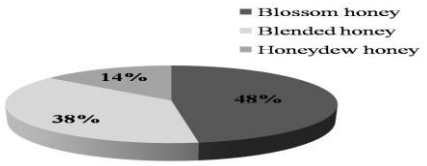

Fig. 1: Types of honey according to Electrical Conductivity (EC) values

Table 2: Physicochemical Properties of Honey Samples

S. No.	Honey Sample	pH Values ± SD	Free Acidity (meq/kg) ± SD	Moisture content (%) ± SD	Electrical Conductivity (mS/cm) ± SD	HMF contents (mg/kg ± SD)	Invertase activity (U/kg ± SD)
1.	H1	3.6 ± 0.2	38.5 ± 1.32	23.06 ± 0.23	0.46 ± 0.0057	55.68 ± 0.65	28.23 ± 3.94
2.	H2	3.7 ± 0.06	38 ± 2.5	21.2 ± 0.4	0.27 ± 0.0057	155.38 ± 2.39	8.31 ± 0.24
3.	H3	3.55 ± 0.04	41.66 ± 1.52	25 ± 0	0.36 ± 0.01	47.1 ±0.56	48.89 ± 1.59
4.	H4	3.86 ± 0.04	47.5 ± 2.5	25 ± 0	1.04 ± 0.0152	139.42 ± 1.64	11.7 ± 0.75
5.	H5	3.73 ± 0.21	57 ± 2.64	23.6 ± 0.4	0.67 ± 0.0057	69.15 ± 0.97	22.67 ± 1.23
6.	H6	3.32 ± 0.03	34.16 ± 0.76	17.33 ± 0.23	0.36 ± 0.0152	155.08 ±13.22	8.1 ± 0.84
7.	H7	3.74 ± 0.03	39.83 ± 0.76	22.4 ± 0.69	0.71 ± 0.0115	204.71 ± 5.3	1.4 ± 0.11
8.	H8	3.91 ± 0.01	27 ± 1.80	18.86 ± 0.11	0.35 ± 0.0057	280.65 ±16.73	1.32 ± 0.55
9.	H9	4 ± 0.03	28.66 ± 1.25	19 ± 0	0.69 ± 0.01	101.19 ± 9.6	18.59 ± 1.27
10.	H10	3.75 ± 0.04	31.83 ± 2.75	15.86 ± 0.11	0.77 ± 0.0057	169.9 ± 0.65	8.04 ± 0.48
11.	H11	3.29 ± 0.01	37.83 ± 2.02	18.8 ± 0	0.47 ± 0.01	439.41 ± 5.76	0.741 ± 0.40
12.	H12	4.1 ± 0.01	35.5 ± 1.80	21.06 ± 0.23	0.96 ± 0.0057	642.31 ± 0.69	0.476 ± 0.16
13.	H13	3.77 ± 0.01	47.66 ± 1.75	21.53 ± 0.11	0.15 ± 0.0057	204.58 ± 6.37	2.64 ± 0.28
14.	H14	3.96 ± 0.02	63.33 ±10.40	20.33 ± 0.11	1.08 ± 0.0057	473.29 ± 1.2	0.74 ± 0.56
15.	H15	3.22 ± 0.02	45.16 ± 1.25	17.33 ± 0.23	0.77 ± 0.01	68.86 ± 0.9	28.2 ± 1.33
16.	H16	3.72 ± 0.02	31.16 ± 1.25	19.86 ± 0.23	0.45 ± 0.0057	144.1 ± 1.64	9.58 ± 1.44
17.	H17	3.99 ± 0.01	47.66 ± 2.51	20.66 ± 0.23	0.64 ± 0.01	113.76 ± 3.89	9.74 ± 1.83
18.	H18	3.12 ± 0.02	44.5 ± 1.80	22.26 ± 0.23	0.80 ± 0.0057	269.48 ± 2.98	1.48 ± 0.24
19.	H19	3.33 ± 0.02	37.33 ± 1.25	20.13 ± 0.23	0.23 ± 0.0057	62.47 ± 1.16	27.01 ± 0.99
20.	H20	3.99 ± 0.05	40 ± 1	19.06 ± 0.23	0.16 ± 0.0057	193 ± 51.9	2.64 ± 0.09
21.	H21	3.67 ± 0.01	43.66 ± 1.04	18.93 ± 0.23	0.60 ± 0.0057	180.03 ± 1.92	3.97 ± 0.99

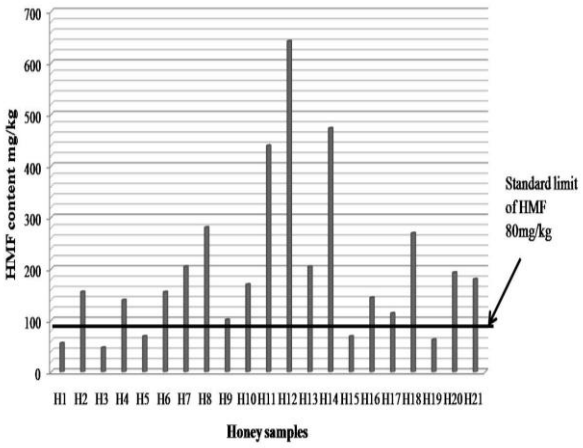

Fig. 2: HMF content of the honey samples with international standard limit.

Statistical analysis

Product moment correlation test between HMF content and invertase activity showed strong negative correlation i.e. -0.6583077. This confirms that, those two parameters are inversely proportionate to each other.

One-way ANOVA of both HMF content and invertase activity suggest statistically significant grouping of the honey samples and these two parameters are directly related to the storage of the honey.

Statistical analysis of other parameters such as, pH, free acidity, moisture contents and EC values were also done but no significant result came out with the groups suggesting no correlation of the parameters on the storage duration of the honey.

DISCUSSION

In the present study, grouping of honey samples (Table 1) on the basis of their storage duration suggests a strong correlation with HMF content and invertase activity which has been proved by statistical analysis. The Group1 comprises of 5 honey samples (H1, H3, H5, H15 and H19) showed low HMF values (47.1 ±0.56 to 69.15 ± 0.97 mg/kg.) within the standard limit (80 mg/kg.) along with invertase activity of 48.89 ± 1.59 to 22.67 ± 1.23 U/kg. The Group 2 comprises of 7 honey samples (H2, H4, H6, H9, H10, H16 and H17) having a high HMF value (169.9 ± 0.65 to 101.19 ± 9.6 mg/kg.) and low invertase activity (18.59 ± 1.27 to 8.04 ± 0.48 U/Kg.)..

The Group 3 with 6 honey samples (H7, H8, H13, H18, H20 and H21) showed the higher HMF value of 280.65 ± 16.73 to 180.03 ± 1.92 mg/kg. and lower invertase value of 1.32 ± 0.55 to 3.97 ± 0.99 U/kg. Finally, the Group 4 consisting of 3 honey samples (H11, H12 and H14), exhibited the highest range of HMF content values (642.31 ± 0.69 to 439.41 ± 5.76 mg/kg.) with minimum invertase activities of 0.74 ± 0.56 to 0.476 ± 0.16 U/kg. The one-way ANOVA for both HMF contents and Invertase activity values showed a p-value of <0.001. This suggests that the grouping of the honey samples is statistically significant at 0.001% level. For the other parameters, like pH, free acidity, moisture contents and electrical conductivity the one-way ANOVA was also performed and was found to have no significant results, thus proves that such parameters are not influenced by storage of honey.

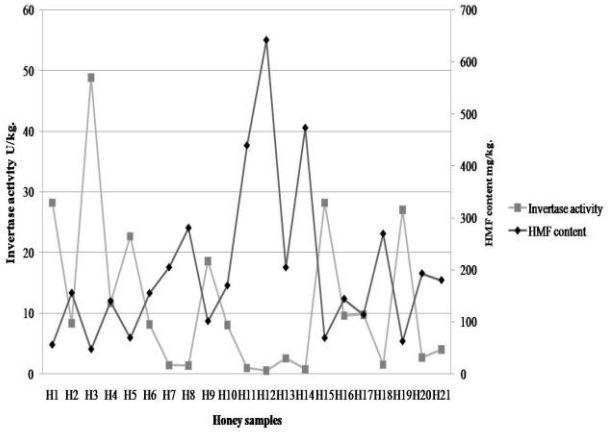

Fig. 3: Comparative study of HMF content and invertase activity of honey samples

Fresh honey should be enriched with invertase and must be devoid of HMF. High temperature enhances decomposition of invertase which begins with 35° C temperature [4]. The rate of decomposition of invertase could be easily obtained during summer and even more in tropical countries like India. In the present investigation, such high amount of HMF content in most of the honey samples was attributed to the storage of honey in natural hot climate. The present study corroborates with the report of Sudan honey where the range of HMF was very high [1]. Hence, in temperate countries, HMF contents are found to be within the recommended limit.

CONCLUSION

The present study reveals the quality of some Indian honey samples on the basis of some important physicochemical parameters mainly emphasizing on HMF content and invertase activity and their impact on storage duration. The studied 21 honey samples showed a great variation in HMF content and invertase activity. The affinity of honey samples toward high HMF values denotes high temperature of the country. So, it is important to impose limits for HMF content in the honey for hot climatic countries like India and it is suggested that honey should be stored at temperature below 35ºC.

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