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Effect of storage on various honey quality parameters of *Apis mellifera* honey harvested from Kannad region, Aurangabad

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Abstract
The present study deals with the effect of storage on *Apis mellifera* honey quality parameters harvested from Kannad taluka of Aurangabad district (M.S.), India. Honey quality parameters like pH, electrical conductivity, moisture content, proline content, HMF content, invertase and diastase activity were measured by AOAC method (2000) at intervals of each six month for the period of 24 month. Student's T-Test was applied to the analyzed data. Results clearly indicates that the pH, electrical conductivity, moisture content and HMF content were increased with the increasing of storage period and concentration of proline, invertase and diastase number were decreased with the increasing of storage time limit. The said physicochemical parameters of honey sample were found statistically significant according to their storage time limit. The obtained data also clearly demonstrate that the invertase is more sensitive than the diastase and HMF content to the storage as well as heat treatment of honey.

Keywords: honey, physicochemical parameters, storage period, invertase, diastase activity, HMF content, etc.

Introduction
Honey is defined as a naturally occurring sweet substance produced by honeybees from the nectar of plants or from secretions of living plants parts. Honey bees collect and transform these raw materials and combine them with specific substances of their own and deposit, dehydrate and store honey in honeycombs to ripen and mature [1]. It is also known as the concentrated aqueous solution of invert sugar. Honey makes complex chemical composition, because it contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments waxes as well as pollen grains [2, 3].

Honey is generally evaluated by a physicochemical analysis of its own constituents. These constituents include the storage quality, granulation, texture, flavor and the nutritional quality of the honey are of great importance to the honey industry, which are also responsible for the medicinal quality of honey. The International Honey Commission (IHC) has therefore proposed certain constituents as quality criteria for honey. These constituents include moisture content, electrical conductivity, reducing sugars, sucrose content, minerals, free acidity and hydroxymethylfurfural [4].

Following are the parameters that can be responsible for the freshness of honey, depends on their normal range which is set by Codex Alimentarius standards [5, 6] as well as Europian Union Honey Directive [1].

pH is an important parameter during the extraction and storage of honey. It influences honey texture, stability and shelf life [7]. In general, a low pH of honey inhibits the growth and proliferation of microorganisms. According to Codex Alimentarius standards [5, 6], the normal range of pH in honey is 3.4 to 6.1. If the pH is increases or decreases above or below the normal range, then several undesirable changes occurs in honey and therefore the honey is not suitable for human consumption.

The electrical conductivity is related to the concentration of organic acids, proteins, and mineral salts in honey [8]. Since the electrical conductivity of honey depends on the flower, in which the bee receives nectar from flowers. It is an important parameter for the indication of honey freshness obtained from different flowers [9, 10]. According to Codex Standard [5, 6], the electrical conductivity of honey should be less than 0.8 mS/cm.

Higher moisture content might be cause unwanted honey fermentation during storage and that leads to formation of acetic acid [11]. Conspicuously, the moisture content of honey depends on various factors, such as the harvesting season, the degree of maturity reached in the hive, and

the geographic and environmental factors [12, 13]. Moisture content in honey should not be exceeds than 21% according to international regulations set by Codex Alimentarius standards [5, 6]. The disturbance in normal range can cause the honey unsuitable for mankind in their daily life diet.

Proline is dominant amino acid found in honey, and has been considered as an indicator of quality and freshness of honey [14]. Also its freshness is depends on heat treatment, honey processing and storage time period. The proline content in honey mainly depends on the time that nectar is processed by the bees [15]. Previous studies found that the proline content of honey was associated with its floral source and geographical origin [16]. Moreover, proline content has been used as an indicator of honey ripeness and sugar adulteration when it falls below a value of 180 mg/kg [17, 18]. Therefore, proline content is a critical marker for the authentication of honey quality [19, 20].

In honey, several harmful and toxic compounds such as HMF may be present and they possibly showed their effect when consumed by humans. Several studies have been shown that the compound has adverse effects of causing mutation, toxic genetically and carcinogenic to mice [21] and it also has adverse effects on blood cells. It was also reported that, it induced tumors and colon cancer also [22]. Due to potential toxic effects, HMF is essential for assessing the conformity of honey [22]. Honey quality is significantly influenced by storage time and heating.

HMF content increase in a way that beyond the set limits indicate to the adulteration of honey whether by selling the old honey as a new replacement of honey class with another cheaper one or by adding cheap materials such as syrup corn starch glucose rich and fructose-rich syrup to honey. Other types of honey falsification are feeding bees with sucrose syrup [24]. The increasing concentration of the HMF compound on the limit refers to the increasing age of the honey [25]. HMF content in honey is an important parameter for determining the quality of honey, its age, antioxidant activity as well as its nutritional value [26].

Normally used enzymes to determine the freshness of a honey are diastase and invertase. Invertase is more sensitive than diastase to thermal treatment and storage period of honey; hence invertase is better parameter to characterize heat treatment and storage time [27]. Improper storage of honey under ordinary conditions causes the deterioration of some useful compounds as well as the accumulation of certain unwanted compounds which lessen its quality, thus making it a less profitable commodity from a commercial point of view [28].

Therefore, the aim of present study is to find out the extent of physicochemical changes which take place during storage period in the honey leading to the variations in its composition.

Materials and methods

Study area: The total area of Aurangabad district is about 10.07 lakh hector is out of which 8.12 lakh hector is under agriculture and 0.12 lakh is forest area. Geographically, Kannad taluka of Aurangabad district is located at 20º 27' N 75º 13' E. The average altitude of this area is 633 meter above sea level. Honey samples were collected from three different locations of Kannad taluka of Aurangabad district.

Collection of sample: 1.5 kg of honey sample of *Apis mellifera* obtained from Kannad taluka was stored under room temperature in closed capped bottle. At intervals of each six month, 150 gm sample were taken for physicochemical analysis for a period of 24 month.

In this study, the pH was measured by using pH meter (pHep, pocket sized Hanna instrument, Portugal), electrical conductivity was measured by conductivity meter (HI96301-2, Hanna instrument, Portugal), moisture content was measured by refractrometer, Hydroxymethylfurfural content (HMF) was determined as per spectrophotometric method described by [29] White (1979), proline was estimated by using ninhydrin method [30], diastase and invertase activity was determined as per method described by [31] Schade (1958), [32] Siegenthaler (1977) respectively. The data was analyzed by applying Student's T-Test.

Results and Discussion

In the present study storage effect of honey on their physicochemical parameters and enzyme activity were studied with regular four intervals of six months in Table No. 1 and Fig. No. 1, 2, 3, 4, 5, 6 and 7.

Among the different physicochemical parameters, hydroxymethylfurfural (HMF) content, diastase activity and invertase activity of honey are the important parameters to determine honey freshness and the heating or storage condition effects on honey quality. HMF (5-hydroxymethyl-2 furfuraldehyde) is an aldehyde and a furan compound which is formed after thermal decomposition of sugars and carbohydrates. The presence of HMF is found to be in many food products like honey, fruit juice, syrup, jam etc. [33].

The HMF plays an intermediate form to produce carcinogenic product but the toxicity of the substance is little known [34]. 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 [35]. High HMF content indicates deterioration of honey which mainly due to unsuitable conditions during storage or heating of honey [25]. The most parameters used as indicators of freshness and overheating of honey are HMF, diastase and invertase.

In the present study storage effect of honey on their quality parameters and enzyme activity were studied with regular four intervals of six months.

I. pH

Most honey samples are supersaturated solutions of fructose and glucose with low pH between 3.2 and 4.5. This relatively acidic pH level prevents the growth of many bacteria. This value could be due to the presence of some weak organic acids primarily gluconic acids, ascorbic acid and even acetic acid [36, 37, 38]. Honey pH values are of great importance during extraction and storage as they influence texture, stability and shelf life [39].

The pH of honey increased significantly with the increase of storage period. The mean value of fresh honey was 3.4 which increased to 5.3 after 24 months of storage of honey. The pH of honey sample was increased 0.3 in first 6 month and 0.6, 1.4 and 1.9 in 12, 18 and 24 months respectively. The results are in agreement with the findings of [40] Fasasi (2012) who reported that the pH of honey is in the range of 3.3 to 4.3 and also agree with the results obtained by [41] Chakraborti and Bhattacharya, (2014) who reported increase in pH from 3.33 to 4.1 with storage. Results clearly indicate that values of pH increased with increasing storage period of honey.

II. Electrical conductivity

Electrical conductivity of the honey depends on the ash, organic acids, proteins, some complex sugars and polyols

content, and varies with botanical origin. The value of electrical conductivity changes, when the amount of the plant pollen decreases [42]. The electrical conductivity of the honey is closely related to the concentration of mineral salts, organic acids and proteins; it is a parameter that shows great variability according to the floral origin and is considered one of the best parameters for differentiating between honeys with different floral origins [43].

The electrical conductivity of honey increased significantly with the increase of storage period. The mean value of fresh honey was 0.29 mS/cm which increased to 0.98 mS/cm after 24 months of storage of honey. Electrical conductivity of honey sample was increased 0.22 mS/cm in first 6 month and 0.39, 0.55 and 0.69 mS/cm in 12, 18 and 24 months respectively.

The obtained results are in agreement with the findings of [28, 41, 44] Qamer *et al*., (2009), Qamer *et al*., (2013) and Chakraborti and Bhattacharya (2014) who reported that the electrical conductivity of honey increased with increase the storage duration. Results clearly indicate that values of electrical conductivity increased with increasing storage period of honey.

III. Moisture Content

The moisture content is an important criterion for evaluating the grade of ripeness of the honey and its shelf-life. In general high amount of water causes the honey to ferment, to spoil and to lose flavor, with ensuing honey quality loss. Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year. High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. During storage of honey, the undesirable fermentation is caused by the action of osmotolerant yeasts resulting in formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water resulting in a sour taste [45, 46].

The moisture content of honey increased significantly with the increase of storage period. However, the values of moisture content of honey after 6, 9, 12 and 24 months, of storage varied critically and were found to be non-significant. The mean value of fresh honey was 18.1 % which increased to 20.00% after 24 months of storage of honey. Moisture content in honey sample was increased 0.2% in first 6 month and later on 0.8, 0.15, 1.9% in 12, 18 and 24 months respectively.

The results of the present study of moisture content are in agreement with the findings of [40] Fasasi (2012) who reported that the moisture content in ranges of 17.5-18.4%. Also supports the findings of [41] Chakraborti and Bhattacharya, (2014) and [47] Evahelda *et al*., (2017) who reported increase in moisture content from 20.13 to 21.06% and 24.25 to 26.30 respectively with storage. Results clearly indicate that moisture content increased with increasing storage period of honey.

IV. Proline content

Although the protein source of honey cannot be identified as a nutrient, the amino acids in honey are important for the origin of honey. The amino acids found in honey are proline, lysine, phenylalanine, β-alanine, arginine, serine, glutamic acid and aspartic acid [18]. The highest amount of amino acid in honey is proline. A criterion used for the separation of honey from bees fed with sugar syrup and honey from nectar is proline [48].

In the present study, the storage of honey had significant effect on its proline content and the mean value of fresh honey was 1026.23 mg/kg which decreased to 840.12 mg/kg after 24 months of storage of honey. Proline content in honey sample was decreased 27.66 mg/kg in first 6 month and later on subsequently 121.85, 147.56, 186.11 mg/kg in 12, 18 and 24 months respectively.

The results of the present study of proline content are in agreement with the findings of [28, 44] Qamer *et al*., (2009; 2013) who reported that the proline content of honey decreased with increase the storage period. Results clearly indicate that concentration of proline decreased with increasing storage period of honey.

V. HMF content

A cyclic aldehyde compound like hydroxymethylfurfural (HMF) is one of such unfavorable compounds, which is virtually absent in fresh and untreated honey. Although HMF is found in a variety of processed foods; honey is the only food for which there exists a recommendation on the allowable content of HMF. Honey samples that have been heat treated, stored in non-adequate conditions or adulterated with invert syrup found high HMF content [49]. It is a recognized parameter related to the quality of honey [50]. The International Honey Commission (IHC) has stated that after processing or blending, HMF levels shall not exceed 40 mg/kg, unless the honey originates from regions with tropical ambient temperatures, in which the levels shall not exceed 80 mg/kg. HMF is formed by the breakdown process of fructose, is toxic and cancerogenic [51]. Fresh honey contains low amount of hydroxymethylfurfural (HMF) with natural levels of enzymes.

The HMF content of honey increased significantly with the increase of storage period. The mean value of fresh honey was 10.77 mg/kg which increased to 18.96 mg/kg after 24 months of storage of honey. The HMF content in honey sample was increased 0.5 mg/kg in first 6 month and subsequently 1.55, 4.25 and 8.19 mg/kg in 12, 18 and 24 months respectively.

The results are in agreement with the findings of [44] Qamer *et al*., (2009); [40] Fasasi (2012); [27] Hasan (2013); [28] Qamer *et al*., (2013); [41] Chakraborti and Bhattacharya, (2014) and [52] Tafinine *et al*., (2018) who reported that the HMF content in honey increased with increase the storage duration. [53] Yilmaz and Kufrevioolu (2001), also reported that the mean value of HMF contents (mg/kg) increased (from 3.3 to 19.1); from 0.00 to 8.8 and from 4.7 to 13.1 after one year storage respectively in their study. The HMF content in the honey sample in the present investigation showed similarly to those mention above. Results clearly indicate that concentration of HMF increased with increasing storage period of honey.

VI. Diastase activity

Saliva of bee is the source of diastase (amylase) that is one of the most important enzymes making honey easily digestible. This enzyme degrades depending on the heat treatment and time applied to honey is a crucial indicator used in the determination of freshness of honey. Enzyme assay in honey can be used to classify a good quality of unadulterated honey [54].

In the present study, the storage of honey had significant effect on the diastatic activity and the mean value of fresh honey was 22.41 DN which decreased to 3.88 DN after 24 months of storage of honey. Diastase activity in honey sample was decreased 2.6 DN in first 6 month and 5.56, 12.19, 18.53

DN in 12, 18 and 24 months respectively. [55] Sahinler *et al*., (2004) reported diastatic activity of honey ranged between1.5-13.7 DN, hence it supports present findings.

However, the results of diastase activity are in agreement with the findings of [44] Qamer *et al*., (2009), [40] Fasasi (2012), [27] Hasan (2013), [28] Qamer *et al*., (2013) and [56] Minhas *et al*., (2016) who reported that diastase activity of honey decreased with increasing the storage period. Results clearly indicate that diastase activity decreased with increasing storage period of honey.

VII. Invertase activity

The storage of honey had significant effect on the invertase activity and the mean value of fresh honey was 26.61 IN which decreased to 0.952 IN after 24 months of storage of honey. Invertase activity in honey sample was decreased 10.82 IN in first 6 month and 17.96, 23.15, 25.65 IN decreased in 12, 18 and 24 months respectively.

The results of the present study of invertase activity are in agreement with the findings of [27] Hasan (2013) who reported that the invertase activity of honey is in the range of 18.7 to 7.8 IN after 8 month of storage and [41] Chakraborti and Bhattacharya, (2014) who reported that the invertase activity of honey is in the range of 27.01 to 0.476 IN. Also [44, 28] Qamer *et al*., (2009; 2013) who reported that the invertase activity of honey decreased with increase the storage duration. 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 [41]. [57] Falllico *et al*., (2009) reported that commercial honey shelf life depends on botanical origin as well as processing. Moreover, data on low diastase honey shows that when only HMF is the quality index, it is a good model to estimate shelf life. The addition to the models of diastase simply increases the uncertainty associated to the shelf life estimation.

Results clearly indicate that invertase activity decreased with increasing storage period of honey. Results also demonstrate that invertase is more sensitive to the heat treatment and storage effect of honey.

In this study honey showed longer shelf life due to natural low HMF contents in 6, 12, 18 and 24 months of storage. Hence raw and unprocessed honey does not pose any serious effect for human consumption in 24 months storage below the 35 ºC. The results also showed that the shelf life of the honey depends on the botanical origin, pH, electrical conductivity, temperature and storage.

Table 1: Effect of storage of honey on various physicochemical parameters.

Parameters	Fresh honey	After 6 month	After 12 month	After 18 month	After 24 month	International Standard Limit (Codex Alimentarius, 2001 and 2019).
PH	3.4±0.11	3.7 ^{NS} ±0.18	4*±0.21	4.8*±0.23	5.3**±0.21	3.4-6.1
Electric conductivity	0.29±0.01	0.51**±0.04	0.68**±0.08	0.84***±0.09	0.98***±0.14	0.8 mS/cm
Moisture content	18.1±0.52	18.3^{NS}±0.57	18.9 ^{NS} ±0.5	19.6*±0.21	20*±0.62	<21
Proline content	1026.23±28.2	998.57 ^{NS} ±25.23	904.38*±19.33	878.67**±21.54	840.12**±17.57	-
HMF content	10.77±0.39	11.27 ^{NS} ±0.43	12.32*±0.38	15.01**±0.83	18.96***±0.72	<40
Diastase Activity	22.41±0.89	19.81^{NS} ±0.92	16.85**±0.74	10.22***±0.71	3.88***±0.25	>8
Invertase Activity	26.61±0.38	15.79**±0.61	8.65***±0.41	3.46***±0.21	0.952***±0.08	6.5-17.7

Mean ± SD, *- *P*< 0.05, **- *P*<0.01, ***-*P*<0.001, NS - Non significant

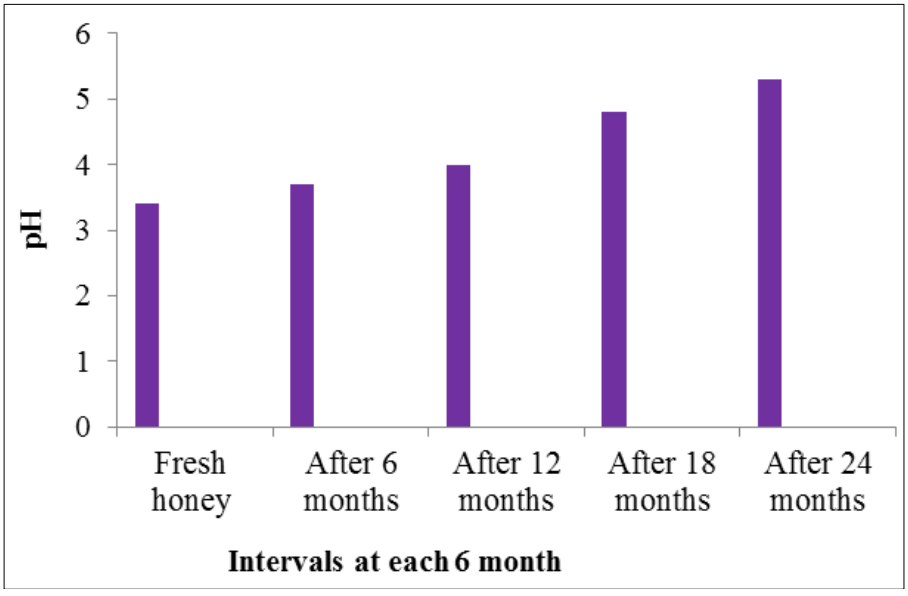

Fig 1: Effect of storage on pH of honey

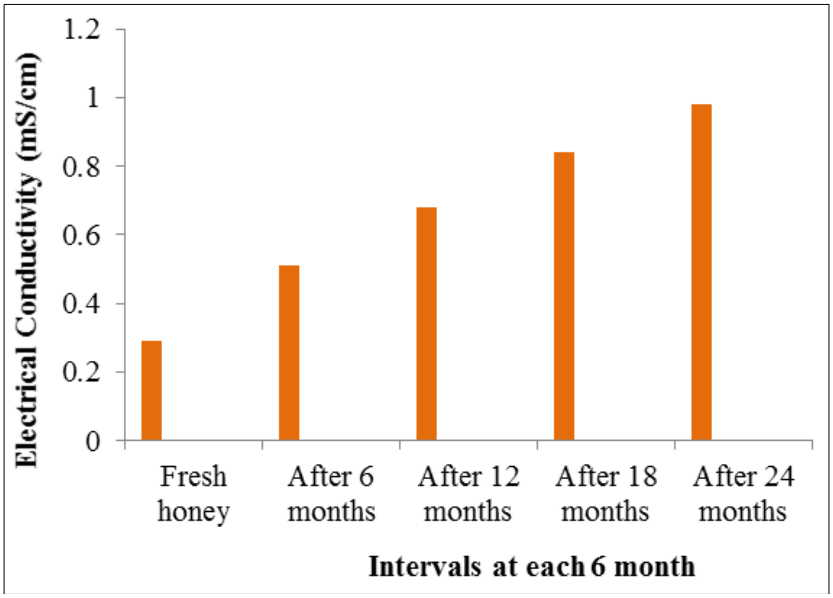

Fig 2: Effect of storage on electrical conductivity of honey

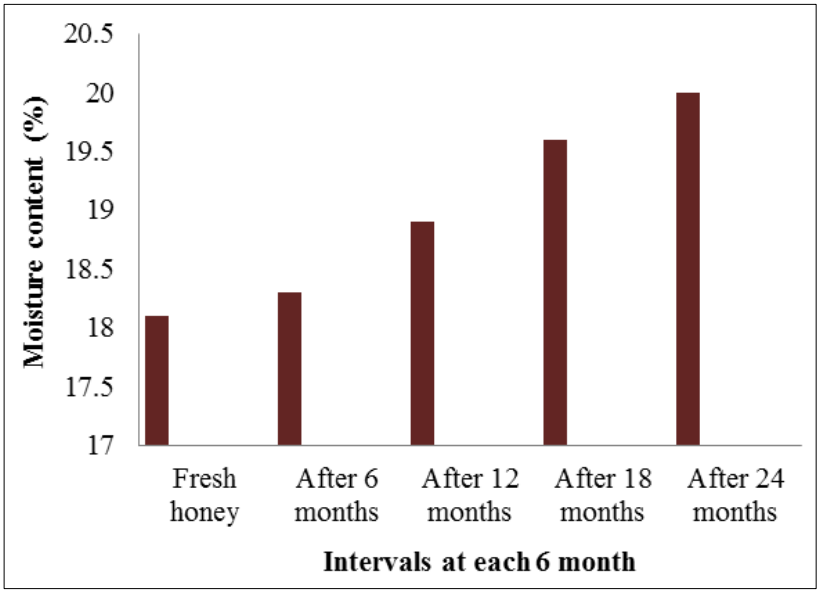

Fig. 3: Effect of storage on moisture content of honey

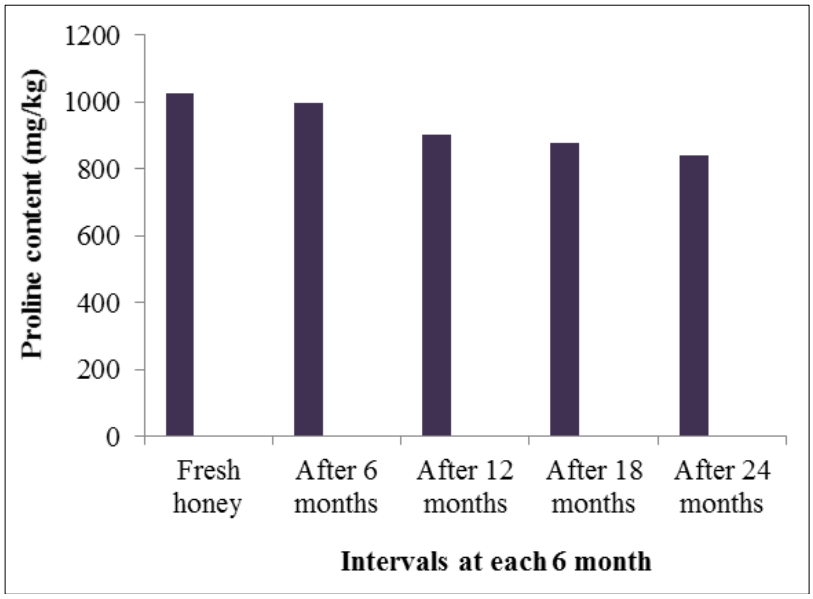

Fig 4: Effect of storage on Proline content of honey

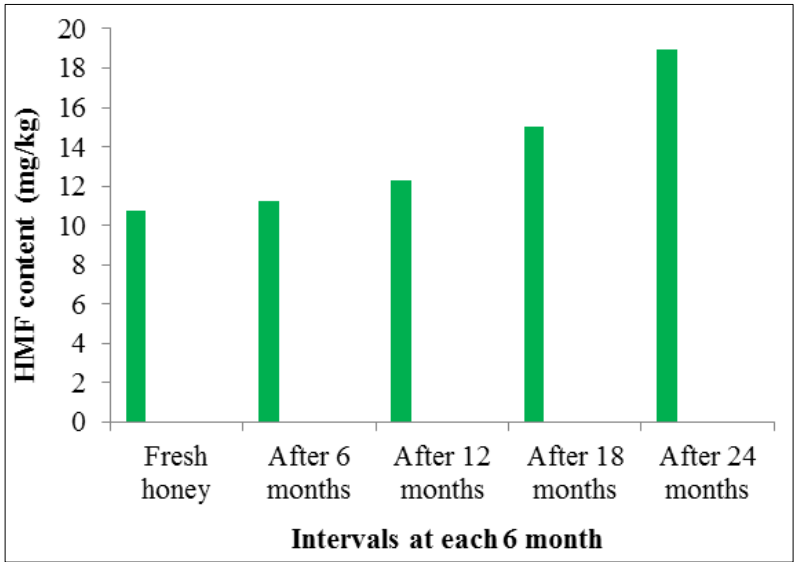

Fig 5: Effect of storage on Hydroxymethyl furfural (HMF) content of honey

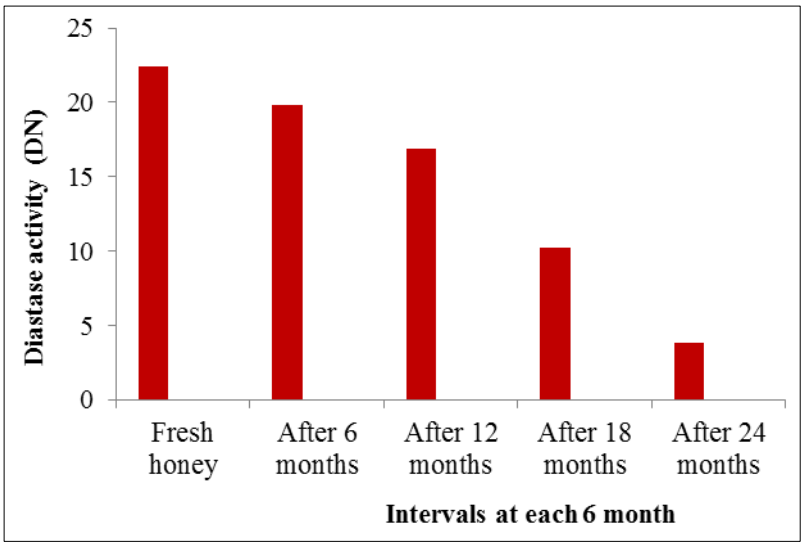

Fig 6: Effect of storage on diastase activity of honey

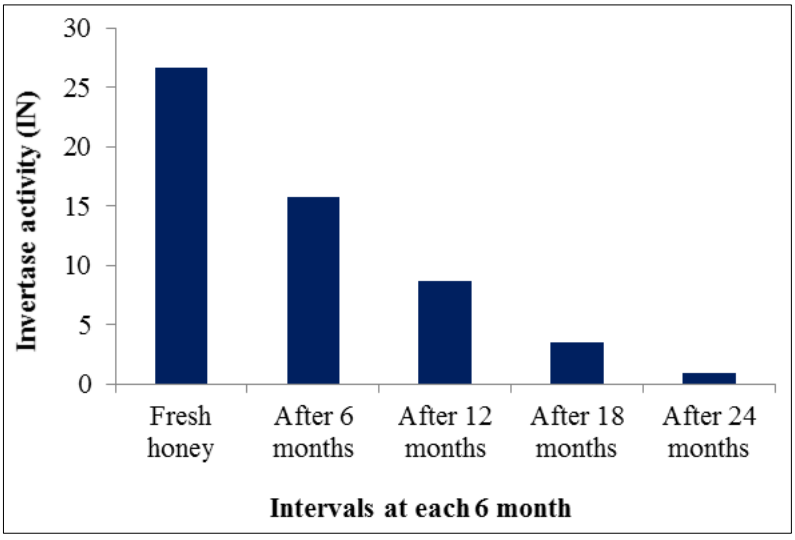

Fig 7: Effect of storage on invertase activity of honey

Conclusion

The physicochemical properties like pH, electrical conductivity, moisture content and HMF content were increased with increasing storage period and concentration of proline, invertase activity and diastase activity were decreased with increasing storage period of honey. Therefore, effect of storage has significantly affected the physicochemical parameters. Increasing the storage period of honey leads into decreasing of its nutritional value. It can be recommended that the honey stored up to 24 months at room temperature can be

safe, suitable for human consumption in their diet of day to day life.

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