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Periodical changes in quality parameters of honey during storage and processing

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Abstract
The effect of storage upto 15 months on apiary honey with an increase in storage period upto 15 months resulted in an increase in the refractive index, Pfund value, TRS, glucose, HMF and yeast and decrease in the moisture, pH and sucrose levels. The properties of honey changed with the advancement of storage. The honey when subjected to indirect heating at different temperatures revealed that moisture content of honey was reduced when heated beyond 50 ^{0}C. A slight variation in TRS content was also observed. In addition to that, the increasing trend in HMF content of honey with the increasing temperature was found. The observation on yeast population in honey exhibited significant growth during the storage. The pollen and yeast counts of processed and unprocessed honey were reduced significantly from 53,760.33 to 38,132.67 and 47,927.33 to 1734.33, respectively.

Keywords: Honey, physico-chemical properties, storage, processing

Introduction
Honey is one of the nature's gift to mankind. Honey is defined as the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of sucking insects feeding on the living parts of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature [1]. The major monosaccharides which contribute to the sweetness of honey are fructose and glucose.

In India, the total honey production per annum increased from 3.7 thousand metric tonnes in 2017 to 4 thousand metric tonnes in the year 2018 [2]. According to National Horticulture Board (2018), among the different states of India, the maximum honey production was from four states namely Uttar Pradesh, West Bengal, Punjab and Bihar, which together account for more than 50 per cent of total honey production in India. The total honey production in Karnataka was approximately 2100 MT in the year 2017-18 [3].

Heat or thermal processing of honey eliminates the microorganisms responsible for spoilage and reduces the moisture content to a level that retards the fermentation process. Raw honey after harvesting is usually strained and filtered to remove suspended materials including pollen, propolis and bee wax prior to heating for commercial processing. Heating honey is to facilitate filtering and bottling of honey by reducing its viscosity [4].

When honey is subjected to mild heat or prolonged storage, a compositional change can occur due to Maillard reaction, caramelization of the carbohydrates and because of decomposition of fructose in the acid medium of honey [5, 6]. The content of free acid, lactones and pH of honey increased slowly with time. Honey darkened with prolonged storage as the contents like fructose, glucose, sucrose, iso-maltose and trisaccharides decreased with time, but contents like maltose and taranose increased on storage [7]. When honey was stored at 23 ^{0} C to 28 ^{0} C for two years, dextrose (13%) and levulose (5%) contents were converted to other forms of carbohydrates [8].

The quality factor of honey like flavour, colour, taste, HMF content and diastase index is strongly influenced by heating and storing duration [9]. There was a decrease in diastase number, invertase content and increase in HMF content, when honey samples were heated at different temperatures, *i.e.*, 55 °C, 65 °C and 75 °C for 5, 15, 20 and 25 min [10].

There was an increase in HMF content with an increase in temperature and pH, whereas diastase activity decreased with increase in temperature [11]. There was a significant decrease in the diastase activity when honey was heated at 85 ^{0}C [12]. Therefore, there is a need to understand the extent of changes in the quality parameters of honey which are responsible for undesirable variations in its composition. Hence the effect of storage and processing on quality parameters of *Apis cerana* and *A. dorsata* honey samples was studied at the Department of Apiculture, University of Agricultural Sciences, Bengaluru, Karnataka, India.

Materials and Methods

Honey samples: Honey samples were collected from the colonies *of Apis cerana* and *A. dorsata* in Apiary and the administrative building (Naik Bhavan) of GKVK, Bengaluru, respectively. The honey was incubated at different temperatures *viz.*, 35 ^{0} C, 50 ^{0} C, 60 ^{0} C and 70 ^{0} C for 15 min in hot water bath and tested for the change in its physicochemical properties such as moisture, TRS and HMF. The apiary honey was stored under room temperature for a period of 15 months and samples were drawn at three months interval for further analysis.

Physico chemical properties: The honey studied for its physico-chemical properties *viz.*, refractive index, moisture, colour, pH, electrical conductivity, ash, total reducing sugars, glucose, fructose, sucrose, HMF and yeast by following the standard methods of AOAC, 1990 [13, 14, 15].

Microbial Studies: Isolation and enumeration of different microorganisms in honey was done by using serial dilution and standard plate count method. The different culture media like Potato Dextrose Agar (PDA) were used for isolating yeast, fungi and and Nutrient Agar (NA) was used for bacterial isolation. The samples were serially diluted upto 10^{-6} dilution. One ml of serially diluted sample of 10^{-3}, 10^{-4} and 10^{-6} was used for isolating yeast, fungi and bacteria, respectively. The respective culture media were poured into plates and incubated at 25-27 ˚C for three days and colony counts were recorded. Microbial counts were expressed as colony forming units per ml of honey (cfu/ml) [16].

Honey storage: The honey was stored under room temperature for periods of 15 months and tested at 3 months interval for its properties *viz.*, refractive index, moisture, colour, pH, electrical conductivity, ash, total reducing sugars, glucose, fructose, sucrose, HMF and yeast.

Honey heating and processing: The honey collected from *A. dorsata* and *A. cerana* combs were subjected to indirect heating at different temperatures *viz.*, 35 ^{0} C, 50 ^{0} C, 60 ^{0} C and 70 ^{0} C for 15 min each in hot water bath and observed for its effect on quality parameters like moisture, total reducing sugars (TRS) and hydroxyl methyl furfural (HMF). The effect of processing was studied by analysing processed and unprocessed honey samples for physicochemical properties.

Results and Discussion

Effect of storage: The effects of storage on physical and chemical properties of honey are presented in table 1 and fig 1. The moisture content of honey showed significant decrease during storage period of 15 months. There was significant variation in the moisture content after 6 months of storage. Initial and final moisture contents were 24.46 and 22.06 per cent respectively. Correspondingly, the refractive index increased at a rate of 0.001 during 0 to 12 months and 0.002, during 12 to 15 months of storage. The moisture content of honey depends on various factors such as harvesting season, degree of maturity reached in the hive and climatic factors [17]. Moisture content is highly important for the shelf-life of the honey during storage because high moisture content which causes honey to ferment and spoil [18].

The colour remained stable for first six months of storage, with light amber color, however, gradually it changed to amber after nine months and then to dark amber in 12 months. Pfund values, corresponding to above months were 79.33, 101.66 and 122.66 mm, respectively. The substances responsible for colour changes in honey are largely unknown. The development of colour in honey is probably due to several factors such as, a combination of tannates and other polyphenols with iron from containers and processing equipment; reaction of reducing sugars with substances containing amino nitrogen (amino acids, polypeptides, proteins); and instability of fructose solution (caramelization) [19]. Darkening of honey could be related with non-enzymatic browning due to Maillard reaction incorporating the sugars and free amino acids or fructose caramelization. These reactions lead to formation of a variety of brown pigments, and simultaneously the formation of intermediate products as HMF. The temperature of storage significantly ($p < 0.01$) influenced colour parameters, however, the room temperature differentiated colour to a greater extent ($\Delta E = 20.37$) than freezing ($\Delta E = 8.07$) [20].

The effect of storage on pH showed that there was a significant decrease at every successive interval of 3 months. The pH was decreased from 4.23 to 3.59 (15.1%). which are in agreement with Ref where they have reported the significant decrease of pH from 4.20 to 3.93 in rapseed honey when stored for 18 months period. pH is a useful index of possible microbial growth. The decreasing trend in pH of honey may be attributed to increase in the acidity, which is imparted to honey due to the production of gluconic acid during its storage. The decrease in pH level in honey was mainly due to gluconic acid production [21, 22, 23]. The pH could be an indicator of possible microbial contamination [24]. Additionally, pH is an important factor during the extraction and storage of honey because it is related to the stability and the shelf life of the product [25].

The total reducing sugar levels in the honey sample increased significantly with storage. This appears to have been contributed by significant increase in glucose content from 33.79 to 37.33 per cent after 15th month of its storage, whereas, the fructose content of honey samples showed a gradual increase upto 9 months of storage period and subsequently showed a decreasing trend for further 6 months. Fructose is supposed to have been accumulated due to break down of sucrose, but it gets converted to HMF. The small decrease in the fructose content of the samples is between 9 to 15 months, probably due to higher conversion of fructose to HMF during that period which is in correlation with continuous decline in sucrose levels and increase in HMF contents during the storage. The increase in TRS may be due to activity of invertase which plays a major role in hydrolysis of non reducing sugars. Several workers reported about the enzymatic activities that lead to hydrolysis of sucrose into glucose and fructose [26, 27, 28, 23].

The HMF content in honey showed rapid and significant changes at each interval of three months in storage from the initial value of 23.16 mg /Kg it reached the highest level of

45.08 mg/Kg after 15 months' of storage. This might be due to the decrease of the fructose content [29]. HMF is a break down product of fructose that increases due to long storage, adulteration and excess of heating. It has been widely reported that, long term effects of heat and storage causes an increase in the HMF content in honey [30]. The increase of the HMF concentration might be to the decrease of the fructose content [29], temperature and time of heating [31, 32, 33], storage conditions, use of metallic containers [32] and chemical properties of honey, which are attributed to the floral source from where the honey has been extracted. The HMF concentrations in fresh Malaysian honey samples was 2.80–24.87 mg/kg, when samples were stored for the duration of 12 to 24 months, they had much higher HMF concentrations that exceeded the recommended levels that are considered to be suitable and safe for human consumption (118.47–1139.95 mg/kg) [34]. However, the samples that were in this study can be safely stored for 15 months, meeting HMF prescriptions.

The yeast population in honey showed significant growth during the storage. The increase in yeast population was significant during tri-monthly intervals from 0 to 3, 3 to 6, 6 to 12 and 12 to 15 months of storage with the respective counts of 5.13, 6.5, 8.5, 9.26, 11.46 and 16.3 cfu/ml. The increase of yeast count is mainly due to growth and multiplication of dormant fungal spores under the favourable conditions of storage.

Effect of heating: There was a significant reduction in the moisture content of honey of both the species as a result of heating (table 2). As the temperature increased from 35 ^{0} C to 70 ^{0} C, the moisture content decreased from 23.0 to 22.10 per cent and 22.0 to 20.17 per cent in honey of *Apis dorsata* and *A. cerana*, respectively. The reduction in moisture content of honey occurred only when heated beyond 50 ^{0} C. As a result of heating there was a slight increase in TRS content of *A. dorsata* honey and decrease in *A. cerana* honey. The conversion of sucrose into monosaccharides could take place during heating which resulted in the variations in sugars. Honey is a supersaturated solution of various sugars. Sugars are the main constituents of honey, comprising about 95% of honey dry weight. The major sugars are the monosaccharide hexoses *viz.*, fructose and glucose, which are products of the hydrolysis of the disaccharide, sucrose. Besides, about 25 different sugars have been detected in honey [14].

Hydroxymethylfurfural (HMF), a cyclic aldehyde, is one such unfavourable compound, which is virtually absent in fresh and untreated honey [35]. 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. HMF is high in honeys that have been heat treated, stored in non-adequate conditions or adulterated with invert syrup [36]. HMF is a recognized parameter related to the quality of honey [37]. HMF content in honey of both the species showed significant changes when they were heated at 35 ^{0} C and beyond till 70 ^{0} C. The heating time and temperature are the main parameters that influence HMF formation in honey [24]. When honey was heated at 65 ^{0} C, the HMF content increased by 14.41 per cent [38, 39]. Heating honey above 75^{o}C is not suitable because it causes degradation of bioactive compounds which could affect the quality of the honey [40].

Effect of processing: Refractive index in unprocessed honey was 1.472 which increased to 1.483 when it is processed, similarly, moisture content was 26% and 21.60%, respectively (table 3). TSS content before and after processing was 71.5^0 Brix and 76^0 Brix, respectively. The colour of the honey depends on pfund scale values in which values are higher in unprocessed and lower in processed honey correspondingly the colour of the honey was changed from darker amber (125mm) to lighter (112mm) before and after processing. The specific gravity of raw honey was 1.357 and 1.39 in processed honey. There was a minor variation in pH of honey before and after processing. The results on electric conductivity showed that processed and unprocessed honey had 0.58 and 0.62 dS/m respectively. Observations on chemical parameters, ash, TRS, glucose, fructose and sucrose content of *Apis cerana* honey showed that there was no significant differences with respect to these parameters between processed and unprocessed honey. Ash (%) was 0.29 and 0.19, TRS (%) was 71.70 and 68.69, glucose (%) was 37.52 and 36.02, fructose (%) was 34.19 and 32.67 and sucrose (%) was 4.01 and 3.52 in processed and unprocessed honey, respectively. HMF content of honey was significantly different between processed and unprocessed honey. There was a gradual increase in its content from35.80 mg/Kg to 44.49mg/Kg. The observations regarding yeast and pollen count per 10 g of honey were showed significant difference between processed and unprocessed. The pollen and yeast counts were reduced significantly from 53,760.33 to 38,132.67 and 47,927.33 to 1734.33, respectively before and after processing.

Thermal treatment applied to honey for different purposes (reducing viscosity, avoiding the crystallization or delay its appearance, destroying the micro-organisms which contaminate) has a significant impact in terms of increase of HMF content [38]. The reduction in moisture is attributed to the removal of moisture by the moisture reduction unit of processing plant under 45 ^{0} C and 15 lbps. Eventually this resulted in increase in per cent TSS, TRS and ash content. The increase in HMF is on account of heating of honey through the pasteurization unit of processing plant at 65^0 C for 10 min. The heat treatment was not only the reason for increase in HMF, some other factors may also affect HMF, like presence of organic acids, moisture content, sugar profile, pH, water activity (aw) and floral source of the honey [41, 42, 43].

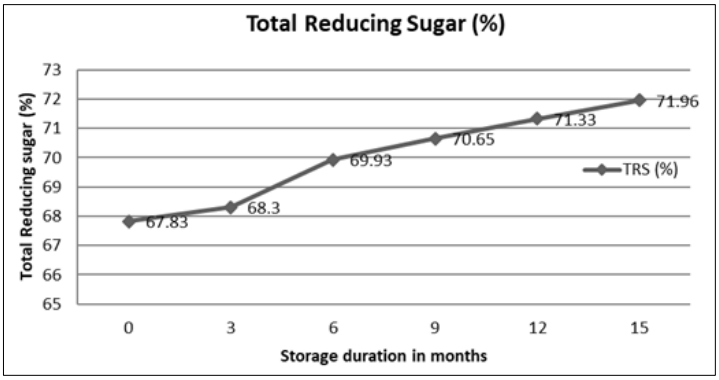
Reduction in EC is mainly attributed to reduction in moisture content. The presence of moisture reduction unit in honey processing plant played a role in reduction of moisture in honey. The number of pollen grains was decreased after honey was subjected to processing plant which is due to filtering of honey through 20 micron filter fitted in the processing plant

The decrease in yeast count and quantity of pollen grains were due to pasteurization. To inhibit yeasts and fungi in natural honey, heating at higher temperature of 80^{o}C for 60s by using the technique of high temperature-short time heating [44]. Heating honey at temperature between 60 to 70^{o}C for 10 min as well as indirect heating in conventional process which is in the range of 60 to 65^{o}C for 25- 30 min can destroy the yeasts completely. The different combinations of temperature and time treatment is necessary to inactivate all types of microbes specially mould and yeasts since they are the only microbes which have been reported to grow in honey [45, 46].

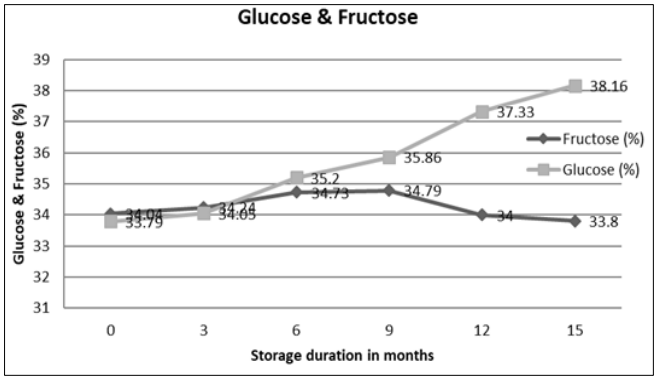
Table 1: Effect of storage of apiary honey on its physical parameters

Storage duration (Months)	RI	Moisture (%)	Pfund (mm)	Colour	pH
0	1.475	24.46	65.33	Light Amber	4.23
3	1.476	24.13	72.66	Light Amber	4.14
6	1.477	24.00	79.33	Light Amber	3.93
9	1.478	23.46	101.66	Amber	3.84
12	1.479	23.06	122.66	Dark Amber	3.75
15	1.481	22.06	150.00	Dark Amber	3.59
SEm±	0.000	0.06	0.83		0.01
CD @ 1%	0.001	0.26	3.62		0.05

RI: Refractive index



(a)



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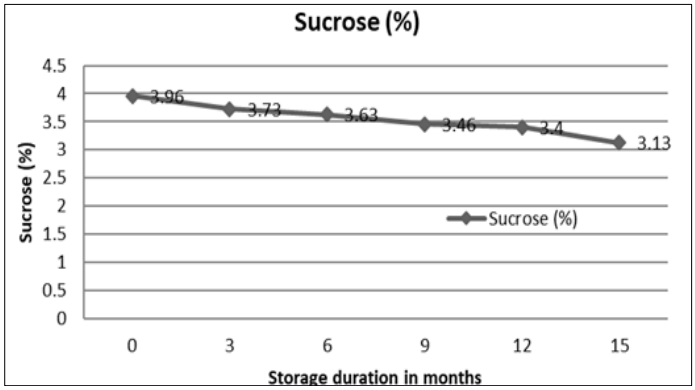

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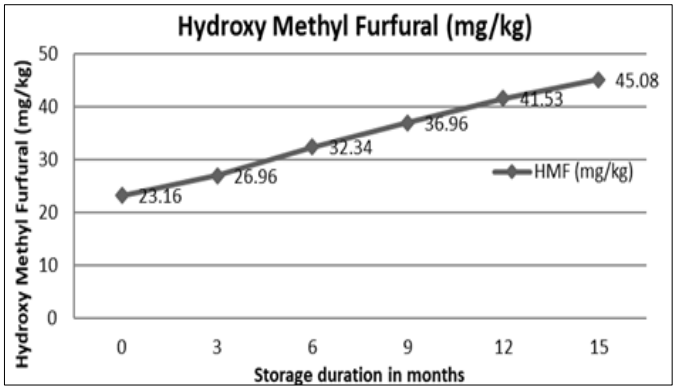

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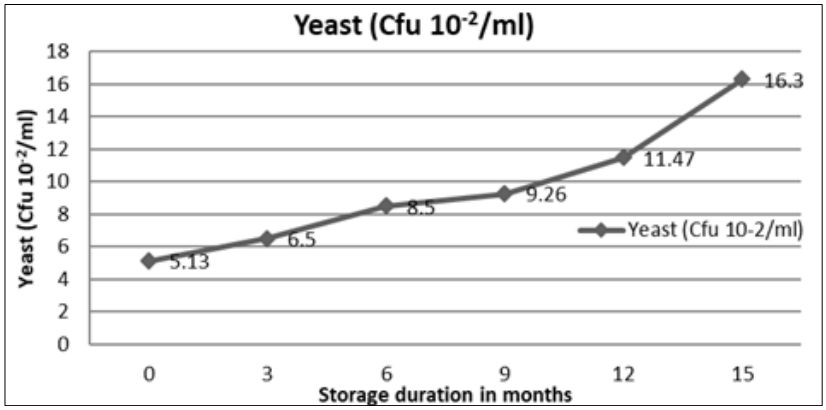

(e)

Fig 1: Effect of storage of apiary honey on its chemical parameters *viz*., (a)Total Reducing (b) Sugars, (c) Glucose & Fructose, (d) Sucrose, (e) HMF and (f) Yeast

Table 2: Effect of heating on quality parameters of honey from *Apis dorsata* and *Apis cerana*

Temperature ^{0} C	Moisture (%)		TRS (%)		HMF(mg/Kg)	
	Apis dorsata	*Apis cerana*	*Apis dorsata*	*Apis cerana*	*Apis dorsata*	*Apis cerana*
Control (29)	23.00	22.00	68.00	70.83	43.93	24.07
35	23.00	22.00	67.67	70.67	44.00	25.00
50	23.00	22.00	67.93	70.33	49.50	29.83

60	22.53	21.13	67.57	70.17	52.73	34.27
70	22.10	20.17	68.33	70.00	64.33	41.50
S.Em±	0.03	0.08	0.33	0.30	0.80	0.76
CD @ 1%	0.13	0.36	NS	NS	3.57	3.39

Table 3: Effect of processing on quality parameters of *A. cerana* honey

Parameters	Statistic tools	Honey from *A. cerana*	
		Processed	Unprocessed
RI @ 20 ^{0}C		1.483	1.472
Moisture %		21.60	26.00
TSS (^{0} Brix)		76.00	71.50
Pfund scale (mm)		112.00	125.00
Colour		Amber	Dark Amber
Specific gravity		1.390	1.357
pH		4.18	4.02
EC (dS/m)		0.58	0.62
Ash (%)		0.29	0.19
	T Cal	2.40 NS	
TRS (%)		71.70	68.69
	T Cal	6.86 NS	
Glucose (%)		37.52	36.02
	T Cal	1.51 NS	
Fructose (%)		34.19	32.67
	T Cal	1.21 NS	
Sucrose (%)		4.01	3.52
	T Cal	0.42 NS	
HMF (mg/Kg)		44.49	35.80
	T Cal	15.77**	
Yeast (per 10 g)		1734.33	47927.33
	T Cal	36.68**	
Pollen (per 10 g)		38132.67	53760.33
	T Cal	14.34**	

RI: Refractive index; TSS: Total Soluble Solids; EC: Electric Conductivity; TRS: Total Reducing Sugars; HMF: Hydroxy Methyl Furfural.
******Indicates significant difference between two means at 1 per cent level of significance

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