ORIGINAL ARTICLE

Effect of Storage on Physicochemical and Biochemical Properties of Unifloral Honey Collected from South India

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ABSTRACT Honey is a natural sweetener, viscous food substance produced from the sugary secretion of the plants (floral nectar) by water evaporation and enzymatic activity. Honey is collected from bee colonies or through bee keeping (apiculture). The physicochemical changes of honey during storage need to be highlighted. In present study unprocessed unifloral honey sample (Imli) collected from apiculture practice was taken for the storage study analyses. The physicochemical parameters such as pH, water activity, specific gravity, moisture content, ash content, colour, browning index, and total solids were analyzed during storage study. pH was found to be decreased during storage study (T0 - 3.71 ± 0.02: T4 - 3.03 ± 0.02). The moisture content was significantly higher at T4 compared to T0 ranging from 19.07 ± 0.04 to 19.38 ± 0.03. The ash content was 0.09 ± 0.01%. Water activity was found to be decreased from 0.6336 ± 0.01 to 0.5912 ± 0.01 during storage study. Specific gravity was increased from 1.4079 ± 0.00 to 1.4274 ± 0.00. The antioxidant (DPPH) percentage was observed to be 63.82 ± 0.30. The total phenolic content was found to be 29.95 ± 1.38 g GAE/Kg of honey. The Biochemical parameter hydroxymethyl furfural was 6.91 ± 0.05 mg/Kg during initial storage and found to be increased significantly after 12 months of storage 79.25 ± 0.83 mg/Kg.

Keywords: Apiculture, Unifloral honey, Storage studies, Physicochemical properties, Hydroxymethyl furfural

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INTRODUCTION

Honey is typically chosen by consumers more for its organoleptic traits than for its nutritional value, with flavour and taste being its most important characteristics. While honey flavour is mostly brought on by honey sugars, the distinctive honey aroma is created by its volatile components, which depend on nectar origin as well as processing and storage conditions (Castro-Vázquez *et al.*, 2008).

The quality of honey could be impacted if the content of the honey changes while it is being stored. It can consequently lower the honey's market value. Data on the effect of storage on different honey varieties is currently limited. Although it is obvious that the lengthy exposure is equally important, variations in the quantities of volatile components during honey storage are thought to be mostly dependent on the temperature to which honey is exposed. There are two main reasons why volatile molecules in heated or stored honey change: compounds that are heat labile and could be destroyed and volatile compounds created by non-

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enzymatic browning (Maillard reaction), which significantly alters honey flavour.

Honey's antioxidant activity increased in temperature conditions and reduced while being stored. Antioxidant activity and honey quality might alter as a result of storage and processing conditions (thermal treatment) (Gheitanchi *et al.*, 2021). Changes in the composition of honey invariably reveal its poor quality and reduce its market value. One of the elements that is crucial for long-term food storage is temperature. By reducing the activity of enzymes and chemical processes and preventing the growth of microorganisms, low-temperature storage can reduce the rate of food degradation. The impact of low temperature on honey has been the subject of numerous studies. However, the most of them are focused on the crystallisation of honey, and only a small number of research examine how compounds' physicochemical qualities and stability have changed (Monggudal *et al.*, 2018).

Although several elements, including as heavy metals (even in minute levels), some alkaloids, and HMF and its derivatives, may increase the toxicity of honey, it is nonetheless regarded as both nutritionally beneficial and therapeutic. HMF is a cyclic aldehyde created by the Maillard reaction, a non-enzymatic browning process, when sugar is degraded during food processing or prolonged honey storage. Honey's simple sugars (glucose and fructose), many acids, and mineral content can all help to increase the creation of this material. Since HMF concentration is often negligible (or present in very minute amounts) in fresh honeys, but tends to increase during processing and/or due to ageing, it is widely recognized as a factor influencing honey freshness (Shapla *et al.*, 2018). Furfural and 5-hydroxymethylfurfural (HMF) concentrations are employed as markers of storage or heating of honeys because it is known that a lengthy period of storage or high temperatures produce furan derivatives through sugar breakdown (Castro-Vázquez *et al.*, 2008).

This study is aimed to find out the changes on physicochemical characters like color, water activity, moisture content, etc., and biochemical properties of imli honey during storage for household purposes, the storage study has been carried for the same samples which are stored in dark at room temperature throughout the study.

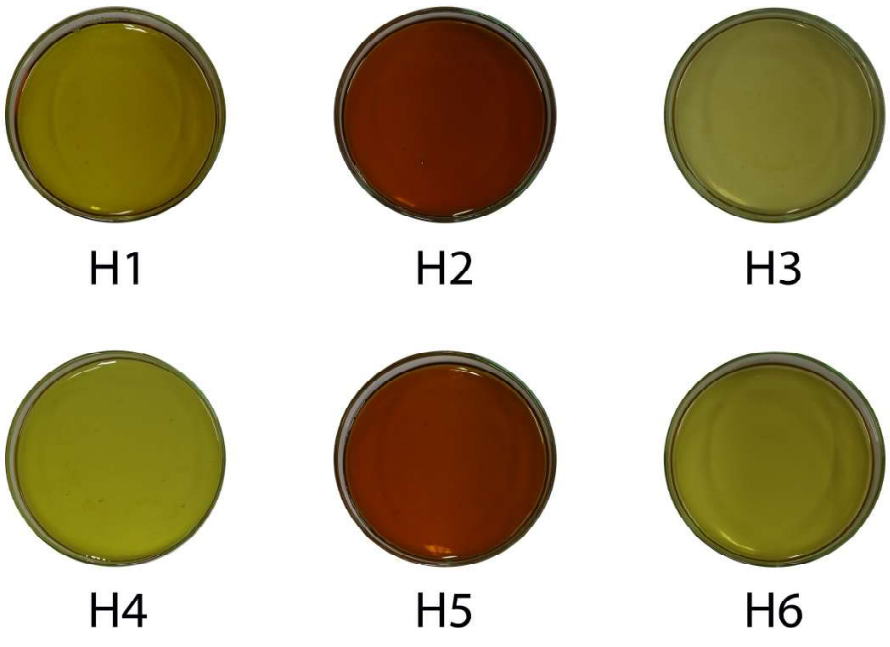
MATERIALS AND METHODS

Sample Collection and Preparation

Fresh, unprocessed unifloral honey sample (H1 – Imli Honey) was collected directly from the apiarist from South India (Aundipatti, Theni, Tamilnadu). The sample was stored at 4 °C until analysis. If the samples were granulated the closed container was placed in water bath without submerging and they were heat at 50-60 °C until liquefied (Food Safety & Standards Authority of India, 2015) before each analysis. The major plant source of the honey is Imli plant *(Tamarindus indica)*. The honey sample was collected from *Apis cerena indica* (Indian honey bee) bee hives (Figure 1).

For the storage study, the honey samples were filled in 100 ml polyethylene bottles, sealed properly and kept in dark

Figure 1: Unprocessed Imli Honey



conditions at room temperature (25 ± 3 ºC). The samples were stored in many bottles for adequate sampling at each time evaluated. Opened bottles were not stored again. The samples were stored for a period of one year (365 days), and the analyses performed at 3 months (90 days) intervals. The parameters total soluble solids (TSS), colour, moisture, pH, specific gravity, water activity and 5-HMF were analyzed 5 times: T0 (Initial 0th day analysis when the samples brought to laboratory); T1 (3 months – 90 storage days); T2 (6 months-180 storage days); T3 (9 months – 270 storage days); T4 (12 months – 365 storage days). Antioxidant activity, total phenolic content and ash content for the sample were performed at T0.

Physico-Chemical Parameters

Ash, specific gravity, TSS, and moisture content of the honey samples were analyze (Food Safety & Standards Authority of India, 2015). For the analysis of the moisture content and TSS, an ATAGO refractometer (Model RX 700, Atago Co., Ltd., Itabashi-ku, Tokyo) was used. All measurements for TSS and moisture content were made at 20 °C, and the results were given in ° Brix and %, respectively. For the analysis of specific gravity, a Pyknometer (25 ml) was utilised. All the analyses were carried out in triplicates.

Water Activity

Using a water activity meter (Model 4TE Aqua Lab Dew Point water activity meter), the water activity (aw) of samples of honey was determined. About 2 g of sample was used to measure the water activity. On the basis of the sample's equilibrium relative humidity, the water activity was determined (ERH). ERH and aw are related by the formula aw = ERH (percentage)/100.(Saxena et al., 2010)

2.2.2. pH

pH meter was used to measure pH of honey samples (LAQUA PH1100 – HORIBA Advanced Techno Co., Ltd. USA) for 10% (W/V) solution [10 gms of honey dissolved in 100ml of water] of honey prepared in mili-Q-water. (AOAC,2012), (Pascual-Maté *et al.*, 2018).

Color Analysis

Using a benchtop color spectrophotometer called the Hunter Lab Color Flex EZ, color characteristics of the honey samples were assessed (Hunter Laboratory Associates, Inc., Reston, Virginia, USA). Hunter Color Lab's guiding idea is to concentrate on the light and measured energy reflected off the material over the whole visible spectrum (Ferrari *et al.*, 2010; and Janghu *et al.*, 2018).

Ash Content

Ash content of honey samples was calculated using a standard procedure described in the International Honey Commission's Harmonized Methods (IHC, 2009). As a result, ashing was carried out in an electric furnace below 600 °C, and residues were weighed.

Biochemical Properties

Total Phenolic Content

The total phenolic content was determined by the Folin – Ciocalteau's method (Janani *et al.*, 2021). About 0.5 g of each honey samples was dissolved in 5 ml of distilled water. From the above solution 0.5 ml of aliquot was fixed with 4.5 ml of deionized water followed by the addition of 0.5 ml of Folin – Ciocalteau's reagent. The solution was mixed thoroughly by vortexing and then 1 ml of 20% sodium carbonate solution was added to the above mixture. Further the above solutions were incubated at room temperature for 1 hr and the absorbance was measured at 725 nm using a spectrophotometer. The total phenolic content was determined by comparing with gallic acid standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (mg GAE)/Kg of honey.

DPPH Radical Scavenging Activity

The scavenging activity of honey samples for the radical 2, 2-di-phenyl-1-picrylhydrazyl (DPPH) was measured using spectrophotometer. 1 g of honey sample was dissolved in 10ml of deionized water. From this 0.5 ml of aliquots was taken and mixed with 1ml of 1 mM DPPH prepared in ethanol. The final volume was made upto 5 ml with ethanol. The ethanol was used as a blank and 1mM DPPH was used as a control. The solutions were incubated at room temperature in the dark for 30 mins and the absorbance was measured at 517 nm. Antiradical scavenging activity was calculated using the following formula: (Janani *et al.*, 2021).

Antiradical activity (%) = (Absorbance of control – absorbance of sample/absorbance of control) x 100 ...(1)

HMF Content

A 50 ml volumetric flask was filled with five grams of honey and had been dissolved in 25 ml of water. Then 0.5 ml of Carrez solution I and 0.5 ml of Carrez II were added, and water was filled in the flask up to the mark. The first 10 ml of the filtrate was discarded after the solution was filtered through filter paper. Two test tubes containing aliquots of 5 ml each were used; one test tube contained 5 ml of distilled water (the sample solution), and the other contained 5 ml of a 0.2% sodium bisulphite solution (reference solution). The absorbances of the solutions were measured at 284 and 336 nm. HMF content was calculated using the below formula (Al-Diab and Jarkas, 2015).

$HMF(mg/Kg) = (A^{-}_{284} - A^{-}_{336}) \times 149.7 \times 5\ XD/W$ ...(2)

where: A_{284} = Absorbance at wavelength 284 nm

A_{336} = Absorbance at wavelength 336 nm

149.7 = Constant

D = Dilution factor (if dilution is necessary)

W = Weight of the honey sample (in grams)

Statistical Analysis

All the analyses were carried out in triplicates. The statistical differences represented by small letters in superscripts were obtained through one-way-analysis of variance (ANOVA) followed by Duncan's multiple range test at 95% of confidence level ($P < 0.05$) using SPSS software 22.0 version.

RESULTS AND DISCUSSION

Physico-Chemical Parameters

Physico-chemical parameters such as moisture, ash content and specific gravity of honey sample during storage were presented in Table 1. The moisture content was ranged from 19.07 ± 0.04 (Initial storage) to 19.13 ± 0.06 (12th month storage). Unifloral honey's moisture content may vary depending on the composition due to varying floral sources, humidity levels, harvesting stage, temperature, etc. The amount of moisture directly affects the stability of honey. The increased moisture content may be due to the several metabolic reactions that may occur during storage in honey (Missio da Silva *et al.*, 2020).

Total soluble solids (TSS) were found to be increased from 79.27 ± 0.06 to 80.33 ± 0.15. Specific gravity was 1.4079 ± 0.00, and 1.4274 ± 0.00. The ash concentration was found to be 0.09 ± 0.00%. Geographical, floral, and meteorological variations may all contribute the variations in ash content (Janghu *et al.*, 2018).

pH

Honey is somewhat acidic, with an average pH of 3.9. This acidity is mainly due to honey's small acid content, which consists primarily of amino acids and organic acids, which gives the distinct flavour. It's also worth noting that honey from tropical countries possess a lower acidity than honey from other parts of the world (Aljohar *et al.*, 2018). The pH values were 3.71 ± 0.02 – for initial storage and 3.03 ± 0.02 after 365 days of storage (Table 2). The fact is that decrease in pH, may indicate microbiological contamination of honey. The results of the present study are consistent with those often seen in fresh honeys (pH = 3.2 to 4.5), which inhibits the most microorganisms growth (Missio da Silva *et al.*, 2020).

Water Activity

The water activity was 0.6336 ± 0.01 (during initial storage) and 0.5912 ± 0.01 (after 365 days of storage) (Table 2). Many microbiological species require water activity values between 0.940 and 0.990 to grow, and most honey samples have a water activity of around 0.600. Yeasts require minimum water activity, between 0.910 and 0.880. While osmotolerant species like *Zygosaccharomyces rouxii* and *Z. bailii* can reproduce at low water activity 0.73 (Kòazovická *et al.*, 2015).

Table 1: Physico-Chemical Properties (TSS, Specific Gravity and Moisture Content) of Unprocessed Imli Honey

Honey No	TSS					Specific Gravity					Moisture Content				
	1st Month	3rd Month	6th Month	9th Month	12th Month	1st Month	3rd Month	6th Month	9th Month	12th Month	1st Month	3rd Month	6th Month	9th Month	12th Month
Imli Honey	79.27 ±0.06^{a}	79.93 ±0.06^{b}	79.97 ±0.06^{c}	80.07 ±0.15^{d}	80.33 ±0.15^{e}	1.4079 ±0.00^{a}	1.4233 ±0.00^{b}	1.4241 ±0.00^{c}	1.4258 ±0.00^{d}	1.4274 ±0.00^{e}	19.07 ±0.04^{d}	18.38 ±0.06^{a}	18.69 ±0.03^{b}	18.95 ±0.06^{c}	19.13 ±0.05^{e}

Note: Results are expressed as mean values ± standard deviations. Means in column with same superscripts are not significantly different ($p<0.05$).

Table 2: Physico-Chemical Properties (pH, Water Activity) of Unprocessed Imli Honey

Honey No	pH					Water Activity				
	1st Month	3rd Month	6th Month	9th Month	12th Month	1st Month	3rd Month	6th Month	9th Month	12th Month
Imli Honey	3.71 ± 0.02^{d}	3.80 ±0.01^{e}	3.43 ± 0.02^{c}	3.27 ± 0.01^{b}	3.03 ± 0.02^{a}	0.6336 ± 0.01^{e}	0.6080 ± 0.00^{d}	0.5945 ± 0.01^{c}	0.5922 ± 0.00^{b}	0.5912 ± 0.01^{a}

Note: Results are expressed as mean values ± standard deviations. Means in column with same superscripts are not significantly different ($p<0.05$).

Color Analysis

Color is one of the most important indicators of honey. Honey with light colour is favored over honey with dark colour. Honey's color is determined by a variety of factors, including flower origin and nectar source. L*, a*, b* (L*—luminosity, a*—from red (+) to green(-), b*—from yellow(+) to blue(-) were the colour parameters analyzed (Scripcã et al., 2019). The L, a, b values were represented in Table 3.

Honey's hue is indicative of its floral origin and is correlated with its mineral, pollen, and phenolic chemical composition. The temperature at which honey is stored and its components have both been linked to honey darkening. Possible reasons for lower L* values include free amino acids, moisture content, nitrogen content, and the fructose/glucose ratio. According to some researchers, the Maillard reaction, the interaction of tannins and other oxidized polyphenols with iron salts, and the instability of fructose in acidic (caramelization) conditions may be the main causes of honey browning (Missio da Silva *et al.*, 2020).

Biochemical Properties

Total Phenolic Content and DPPH Antiradical Scavenging Activity

The total phenolic content of unifloral honey was 29.96 ± 0.15 g GAE/Kg of Honey (Figure 2). Dark honeys have higher total phenol content than light honeys from different geographical sources (Gül and Pehlivan, 2018). Our results were in accordance with the previous studies in which the samples from west Bengal region of India have been analyzed (Das, 2013).

The free radical reagent 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was chosen among the several methods to investigate the antioxidant capacity of natural products. The antiradical scavenging activity was found to be 63.82 ± 0.3% (Figure 2).

HMF Content

The HMF content was found to be increased gradually during storage. Initially it was found to be 6.91 ± 0.05 at T0, 6.4 ± 0.04 at T1, 26.83 ± 0.02 at T2, 45.96 ± 0.38 at T3, and 79.25

Table 3: L, a, b Values of Unprocessed Imli Honey

Honey No	Colour Analysis														
	L					a					B				
	1st Month	3rd Month	6th Month	9th Month	12th Month	1st Month	3rd Month	6th Month	9th Month	12th Month	1st Month	3rd Month	6th Month	9th Month	12th Month
Imli Honey	2.93 ± 0.04^{c}	1.86 ± 0.01^{a}	2.50 ± 0.01^{b}	3.62 ± 0.03^{d}	4.24 ± 0.05^{c}	-0.35 ± 0.08^{a}	0.35 ± 0.03^{c}	0.23 ± 0.06^{d}	0.12 ± 0.03^{c}	0.03 ± 0.02^{b}	2.45 ± 0.06^{a}	2.82 ± 0.01^{c}	2.76 ± 0.01^{b}	3.15 ± 0.04^{d}	3.56 ± 0.04^{c}

Note: Results are expressed as mean values ± standard deviations. Means in column with same superscripts are not significantly different ($p<0.05$).

Figure 2: Total Phenolic Content and Antiradical Scavenging Activity of Unprocessed Imli Honey Sample

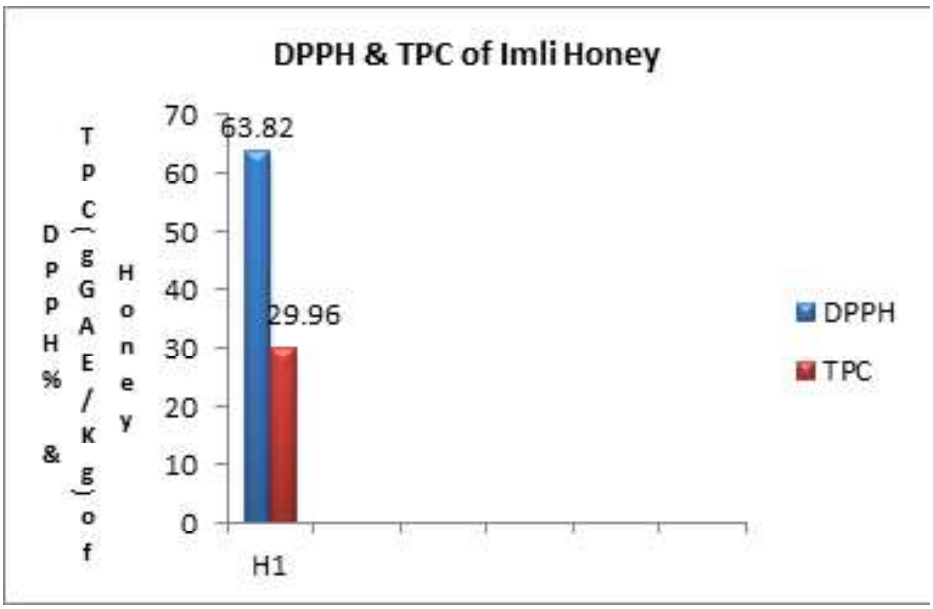
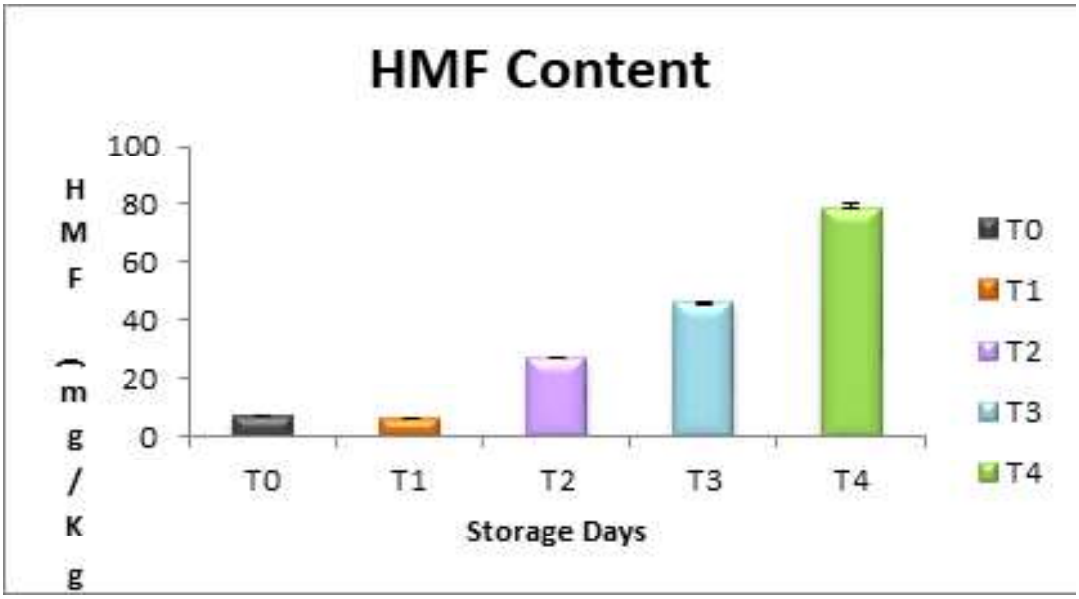


Figure 3: HMF Content (mg/Kg of Honey) of Unprocessed Imli Honey Sample



± 0.83 at T4 (Figure 3). Though the HMF content in honey sample increased significantly during storage it was found to be in limit in accordance with Codex Alimentarius (80 mg/Kg) at T4 i.e., after 365 days of storage. In the work carried out by (Fallico *et al.*, 2008), the honey samples treated at different temperatures were resulted in degradation of HMF compound and they concluded that the levels of HMF in honey could not be so accurate for the evaluation of honey shelf life.

CONCLUSION

The present study established the correlation between the physico-chemical parameters of honey such as water activity, colour, pH, Specific Gravity, TSS, moisture content, etc., during storage. When evaluating the imli honey (*Apis cerena indica*) it was observed that the storage time and temperature influenced the physicochemical parameters. pH was found to be decreased during storage and the water activity, moisture content, specific gravity, TSS found to be increased. The biochemical compound HMF found to be increased along with storage period. Further studies are needed to note down the minimal changes in individual components of flavonoids and phenols and quantification of such components using the high-end instruments like GC-MS (Gas Chromatography Mass Spectrometry) and HPLC (High Performance Liquid Chromatography).

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