



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2021; 9(2): 578-586

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Received: 18-12-2020

Accepted: 05-02-2021

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Physicochemical properties of blended raw honey samples collected from three different locations of Kannad taluka of Aurangabad district (MS), India

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 DOI: <https://doi.org/10.22271/j.ento.2021.v9.i2i.8534>

Abstract

The present study was carried out to investigate the physicochemical properties of blended raw honey samples collected from three different locations from Kannad taluka of Aurangabad district (MS). The parameters like moisture content, pH, electrical conductivity, total reducing sugars, glucose, fructose, fructose glucose ratio, sucrose, Hydroxymethylfurfural (HMF), proline, protein content, Vitamin C, lipid content, were analyzed by AOAC method (2000). The results clearly indicate that the samples compared favorably with samples in many parts of the world and also fall within the limits of international standards. The variations in the physicochemical properties of honey samples are related to differences in the floral sources, climatic conditions and site of collection to the studied area. Overall the results indicate that the nutritional quality of honey was different from species to species and from location to location.

Keywords: Raw honey, Hydroxymethylfurfural, physicochemical properties, floral sources, etc.

1. Introduction

Honey, one of the major bee products, is a sweet viscous natural fluid made from the nectar of plants. Honey was defined as “the sweet substances produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs”^[1]. It is a concentrated aqueous solution of invert sugar that comprises a mixture of other compounds like carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex^[2, 3, 4]. Many scientists reported that natural honey contains about 200 substances, which consist of not only a highly concentrated solution of sugars, but also the complex mixture of other saccharides, amino acids, peptides, enzymes, proteins, polyphenols, organic acids, carotenoid like substances, vitamins and minerals^[5, 6, 7].

The composition of honey varies due to the influence of plants, climate, and environmental conditions as well as the ability of the beekeeper. The alteration of the physicochemical properties of honey depends on the nectar and pollen of the original plant, color, moisture, and protein and minerals contents. Therefore, honey is related to its botanical origin, processing and storage, and climatic factors that occur during the flow of nectar, and to the temperature at which the honey matures in the hive^[8]. Although, the main components of honey are almost identical in all honey, yet the chemical composition and physical properties of natural honey depend on the floral sources, the processing, storage and climatic conditions^[9, 10, 11].

Honey has become one of the most commercial agricultural products in many countries in the world. Honey is the major bee product which has important nutritional value and provides significant economic contributions. Quality control of honey is important to determine its suitability for processing and to boost the demand of the market. Honey shall not have foreign taste, going to ferment, heated to the amount or degree of destroying its natural enzymes and a substance that endanger human health^[12]. The international honey commission (IHC) has proposed certain constituents as quality criteria for honey. These include moisture content, electrical conductivity, reducing sugars, amount of fructose and glucose, sucrose content, individual sugars, mineral, free acidity, diastase activity, HMF content, invertase activity, proline content and specific rotation^[13, 14].

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The physicochemical properties of honey are helpful for the comparison of natural honey samples from different locations and also serve as important indicators that can help to distinguish between natural and artificial honey. The physicochemical properties provide the parameters for the characterization and classification of honey. They also serve as criteria used for choosing appropriate processing and packaging techniques and technological applications of natural honey [15]. Moreover, the apiculture sector has received little research and developmental attention and the honey produced in the different agro-ecologies of the country has not been characterized to date.

To date no study has been conducted to examine the quality and physicochemical properties of honey produced in Kannad taluka of Aurangabad district. To increase the income of beekeepers and the marketability of honey produced in the study area, it is important to determine the physicochemical properties of the honey face to face national and international standards set for honey. The present study deals with the different physicochemical parameters of honey samples found in three different locations of Kannad taluka of Aurangabad district.

2. Materials and methods

A) Collection of samples: Honey samples were collected from three bee species (*Apis florea*, *Apis cerana indica* and *Apis dorsata*) from three different locations of Kannad taluka of Aurangabad district, (M. S.) during October 2015 - September 2016. Total of 23 different honey samples were collected as follows: 9 from an agricultural area, 9 from road side area and 5 from the forest area. Area wise honey samples were blended in equal quantities (100 gram each) and honey samples were put in air tight sterilized plastic containers. They were labeled, brought to the laboratory and stored at 0 - 4° C until analysis.

B) 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 20° 27' N 75° 13' E. The average altitude of this area is 633 meters above sea level. Honey samples were collected from three different locations of Kannad taluka of Aurangabad district.

i. Agricultural area

There are total of 9 samples of honey harvested from the agriculture areas. In which 7 samples of *Apis florea*, 1 sample of *Apis cerana indica* and 1 sample of *Apis dorsata* bee species. The distance between these hives is above 20 meters from the road side. Farmers are engaged in cultivation of traditional crops like sunflowers, mustard, maize, cotton, jowar, pearl millet, pulses, onion, other seasonable vegetables and also cultivating fruit plants like pomegranate, sweet orange etc. in the area provided and independent system in which the bees assist to carry out cross pollination.

This area includes locations viz., Tapargaon, Hatnur, Devgaon (Rangari), Adgaon (Jehur), Pishor, Wasadi and Lohagaon. The distance between the two sampling stations is approximately 1-20 km.

ii. Road side area

There are total 9 samples of honey harvested from road side area. In which 8 samples of *Apis florea* and 1 sample of *Apis*

dorsata bee species were obtained. The distance between these hives is between the 20 meters from the road side.

This area includes locations viz., Tapargaon, Hatnur, Kannad City, Shivrai, Panpoi phata, Andhaner road and Hivarkhedha. The distance between the two sampling stations is approximately 1-5 km.

iii. Forest area

The Gautala forest is situated 8 km away from Kannad Taluka. The forest is famous for woody plants, shrubs, medium size as well as huge trees, lianas and climbers etc. The forest has spread upon Sahyadri hill ranges of Western Ghats. The forest acquired about 260 sq. km area on the boundary of Marathwada and Khandesh. The forest is confined to the Ajanta Satmala ranges in Kannad and Sillod taluka. Geographically it is situated 74°-55° and 75°-15° east longitude and 20°-15° and 20°-30° north latitude [16]. There are total 5 samples of honey harvested from the Gautala forest area. In which 3 samples of *Apis florea*, 1 sample of *Apis cerana indica* and 1 sample of *Apis dorsata* bee species were obtained.

The following wild plants species were found in forest area- *Amaranthus tricolor* (Tandul-kunjra), *Aegle marmelos* (Bel), *Amaranthus spinosus* (Kante bhaji), *Bauhinia purpurea* (Rakta Kanchan), *Carissa carandas* (Karvand), *Coleus barbatus* (Karmelo), *Luffa cylindrica* (Ghosala), *Moringa oleifera* (Shevga), *Oxalis corniculata* (Ambutee), *Prosopis cineraria* (Shemi), *Portulaca oleracea* (Ghol), *Sesbania grandiflora* (Hatga), *Tamarindus indica* (Chinch), *Mangifera indica* (Aamba), *Limonia acidissima* (Kavath), and *Phyllanthus emblica* (Aavla).

C) Physicochemical Analysis

Honey samples were analyzed for pH, moisture, electrical conductivity, total reducing sugars, glucose, fructose, F/G ratio, sucrose, Vitamin C, lipid content, Hydroxymethylfurfural (HMF), Proline content and protein content. All of these analyses were done following AOAC Method (2000) [17].

i. Determination of pH- The pH was measured by means of a pH-meter (pH ep. pocket sized, HANNA Instruments, Portugal. Range – 1-14, accuracy ± 0.1 pH). The pH of a 10% (w/v) solution of homogenized honey prepared in boiled warm water was measured by a pH-meter. The pH meter was calibrated using standard buffers of pH 4.0, 7.0 and 9.0 prior to measuring the pH of the samples.

ii. Determination of Electrical Conductivity- The Electrical Conductivity was determined by a conductivity meter (Model no. HI96301-2, Range 0-19900µS/cm, Accuracy±0.2%, HANNA Instruments, Portugal). Electrical Conductivity meter was first calibrated with water and then conductivity meter was dipped into honey Solution (10.0%) and reading was noted after stabilization of instrument.

iii. Determination of Moisture Content- Moisture content of honey samples were determined by using refractometer reading at 20°C and obtained corresponding percentage moisture from AOAC standard table. Moisture content was determined by using refractometer (Model no. ZL03303431.1, Pal-3, Atago pocket refractometer Japan). The refractometer was calibrated by adjusting zero. After that 2-3 drops of

honey was put on the prism and reading was noted in the record book for all honey samples.

iv. Determination of Vitamin C by Colorimetric Method

Extraction: Take 1 gm of honey in 10 ml of 4% oxalic acid solution in conical flask. Add bromine water dropwise with constant (the enolic hydrogen atoms in ascorbic acid are removed by bromine). When the extract turns orange yellow due to excess of bromine, expel it by blowing in air. Make up to a known volume 25 ml with 4% oxalic acid solution. Similarly, convert 10 ml stock ascorbic acid solution into dehydro form by bromination.

Estimation: Pipette out 10-100 μ g standard dehydroascorbic acid solution into a series of tubes. Similarly pipette out different aliquots (0.1-2ml) of brominated samples. Make up the volume in each tube to 3 ml by adding distilled water. Add 1 ml of DNPH reagent followed by 1-2 drops of thiurea to each tube. Set a blank as above but with distilled water in place of ascorbic acid solution. Mix the content of the tube thoroughly and incubate at 37°C for 3hrs. After incubation dissolved the orange-red osazone crystals formed by adding 7 ml of 80% sulphuric acid. Absorbance was measured at 540 nm. Plot a graph of ascorbic acid concentration versus absorbance and calculate the ascorbic acid content in the samples.

v. Determination of HMF content: HMF was determined by Spectrophotometric method White, (1979) [18] after clarifying samples with potassium hexacyanoferrate (Carrez I) and zinc sulfate -7- hydrate (Carrez II) and the addition of sodium bisulphate. Absorbance was determined at 284 nm and 336 nm in Elico Biospectrophotometer BL 200.

Five grams of honey were dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, added by 0.5 ml of Carrez solution I and 0.5 ml of Carrez II and make up to 50 ml with water. The solution was filtered through paper rejecting the first 10 ml of the filtrate. Aliquots of 5 ml were put in two test tubes; to

one tube was added 5 ml of distilled water (sample solution); to the second was added 5 ml of sodium bisulphite solution 0.2% (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using Elico Biospectrophotometer BL 200.

vi. Determination of Protein content: The protein content of honey was measured according to Lowry *et al.*, (1951) [19]. Briefly, BSA solutions were prepared by diluting a stock BSA solution (1 mg/ml) to 5 ml. BSA concentrations ranged from 0.05 to 1.00 mg/ml. Based on these different dilutions, 0.2 ml of protein solution was placed in different test tubes and 2 ml of alkaline copper sulfate reagent (analytical reagent) was added. After the resulting solution was mixed properly, it was incubated at room temperature for 10 min. Then, 0.2 ml of reagent Folin-Ciocalteu solution was added to each tube and incubated for 30 min. The colorimeter was calibrated with a blank, and the absorbance was measured at 660 nm.

vii. Determination of Total Reducing Sugar Assay- 3,5-Dinitrosalicylic acid (DNSA, IUPAC name 2-hydroxy-3,5-dinitrobenzoic acid) is an aromatic compound that reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm (In case of glucose).

Procedure

Take at least three 20 ml test tubes (i.e. three replicates of each concentration should be tested) and took an amount of glucose stock solution in each test tube as per table given below Prepared a blank, in which case added 500 μ l of DW instead of sample. Added DW as indicated in the table above (preheated to 65°C). Incubated precisely at 65°C for 15 min in a water bath or incubator. Add 3 ml of DNSA. Kept tubes (Glucose solution + DW + DNSA) in boiling water-bath for 15 min. Cool at room temperature. Absorbance was measure at 540 nm in a UV Elico spectrophotometer BL-200 against a suitable blank.

Standard for DNSA methods. Sr. No.	Conc. of glucose (μ mol)	Amount of working solution (μ l)	Volume of DW (μ l)	Amount of DNSA (ml)
1	1	100	1400	3
2	2	200	1300	3
3	3	300	1200	3
4	4	400	1100	3
5	5	500	1000	3
6	-	-	1500	3

viii. Determination of Glucose, Fructose and Fructose

Glucose ratio- Glucose percentage is determined iodometrically in a weak alkaline medium and the value is subtracted from reducing sugar percentage to arrive at fructose percentage and fructose: glucose ratio.

Glucose% = Normality of sodium thiosulphate solution \times (B-S) \times 0.009005 / (0.1N \times weight of sample) \times 100

Fructose % = Reducing sugars% - glucose%

Fructose Glucose ratio = Fructose% / Glucose%

ix. Determination of Sucrose content: The sucrose content of the honey samples was determined according to the procedure of Lane and Eynon method (1923) [20].

2.6 g of honey were weighed and then transferred to a 500 ml volumetric flask. Five milliliters of standardized Fehling A and B solutions were transferred to a 250 ml

Erlenmeyer flask, with 7 ml of water and 15 ml of honey solution. The Erlenmeyer flask was heated and 1 ml methylene blue 0.2 % was added. Titration was carried out adding the diluted honey solution until the indicator was decolorized. Determining sucrose content was carried out by inversion, adding 10 ml of diluted HCl, 50 ml diluted honey solution and water to a 100 ml volumetric flask, heating in water bath, then cooling and diluting to mark. Finally the Lane-Enyon method was applied and sucrose content was obtained by difference.

Apparent sucrose = (Invert sugar / 100gm honey after inversion) - (Sugar content before inversion) \times 0.95.

The results were expressed as gm apparent sucrose/100 gm honey.

x. Determination of Proline content: Proline was determined by Ninhydrin method and results were recorded at 520 nm using a spectrophotometer. Honey

solution (1g/20ml) was taken in three reaction tubes separately. Formic acid (0.25ml) (Riedel chemical, 85%) and ninhydrin solution (Spectrochem, 99%) (1ml of 3% ethylene glycol monoethylether) (Ranbaxy) were added into it. The tubes were tightly capped, shaken well and placed in boiling water bath for 15 minutes and cooled for 5 minutes at room temperature. Caps were removed and 5ml of aqueous isopropyl alcohol (50% aq.IPA) (Qualigens, 99%) was added into each reaction tubes. The content of the tubes were mixed well and absorbance was determined at 520 nm, using the spectrophotometer (Elico, Model no.-BL 200). Absorbance of all samples was noted within the 35 minutes of cooling. Calibration curve plotted with standard solution of proline against absorbance. Proline in honey was calculated from standard curve.

- xi. Determination of Lipid content-** Lipid content was determined by Bligh and Dyer (1959) method [21]. Homogenize the sample 20g with 16 ml distilled water, 40ml of chloroform and 80 ml of methanol at the speed of 9500rpm for 1min at 4degree C. Add 40ml of chloroform and homogenize for 30seconds. Add 40 ml of distilled water and homogenize again for 30seconds. After centrifugation of the homogenate at 2000rpm at 4degree C for 20min transfer the supernatant in to a separatory funnel and allow it to separate. Determine lipid content gravimetrically by measuring triplicate aliquots of the chloroform layer into tared containers, evaporate the solvent and weigh. Calculate the lipid content.

3. Results and Discussion

The results of different parameters of all the raw blended honey samples collected in three different locations of Kannad taluka were compared with the Codex Alimentarius and European Standards [12]. It was observed that studied physicochemical parameters are within the normal ranges (Table 1 and 2).

Honey samples harvested in three different locations of Kannad taluka were obtained and used for the study. All the samples were collected freshly in sterile containers and stored at room temperature until analyzed. Unnecessary materials such as wax sticks, dead bees and particles of combs were removed before analysis.

The mean pH values of blended honey samples obtained from three different locations of Kannad taluka were within the range of 3.8 to 5.3. The pH is an important parameter during extraction and the conservation of honey. It increases the quality, constancy and shelf life of honey [22]. This parameter is related to fermentation process due to storage [23, 24]. It is the helpful index for possibility of microbial growth, because of the presence of various organic acids viz., pyruvic acid, maleic acid, citric acid and gluconic acid in balance equilibrium with lactones, esters and inorganic ions like sulphate, phosphate and chloride [25]. Honey samples with a pH above 5 to be of low quality [26]. The analysis of pH in the honey is considered as one of the quality factors used in the international honey trade [27].

Sajid *et al.*, (2020) comparatively studied the pH of fresh and branded honey from Pakistan in the range of 4.35 to 7.5 in fresh honey and 4.6 to 5.35 in branded honeys [28]. Goncalves *et al.*, (2018) studied the pH of selected Portuguese commercial monofloral honey samples in the range of 3.43-4.18 [29]. Kumar *et al.*, (2018) studied the pH of 100 Indian honey samples ranges from 3.81-4.85 [30]. Boussaid *et al.*,

(2018) studied the pH of six Tunisian honey samples from various floral origins ranges from 3.67-4.11 [10]. Lullah-Deh *et al.*, (2018) studied the pH of seven honey samples from Mambilla Plateau, Nigeria ranged from 3.22 to 5.00 [31]. Silva *et al.*, (2017) reported the pH value of Portuguese honey from Castelo Branco region in the range of 5.53 to 5.33 [32].

Ndife *et al.*, (2014) studied the pH of Nigerian honey sourced from different floral locations ranges from 4.10 to 4.58 [33]. Sohaimy *et al.*, (2015) reported the pH of Egyptian, Kashmiri, Yemeni and Saudi honeys in the range 4.11- 4.63 [34]. Buba *et al.*, (2013) studied the pH of honey samples from North-East Nigeria ranged from 3.5-4.9 [35]. Liberato *et al.*, (2013) reported the pH value of 22 honey samples from Ceara state, Northeastern Brazil ranges from 3.01-4.21 [36].

The mean values of moisture content in blended honey samples obtained from three different locations of Kannad taluka were within the range of 15.96 to 18.23%. The moisture content of honey is an important quality parameter and influenced by various aspects such as maturity period, climatic conditions, harvesting time and type of honey [37, 38]. Higher water content could lead to the fermentation of honey during storage [39, 40]. The water content depends upon the environmental factors during production such as weather and humidity inside the hive, but also on nectar conditions and treatment of honey during extraction and storage [41].

Sajid *et al.*, (2020) comparatively studied moisture content in fresh and branded honey from Pakistan in the range of 18 to 19.07 % in fresh honey and 19.50 to 21.25 % in branded honey [28]. Goncalves *et al.*, (2018) reported the moisture content of selected Portuguese commercial monofloral honey samples ranges between 15.7-16.5% [29]. Kumar *et al.*, (2018) reported the moisture content of 100 Indian honey samples ranges between 18.37-22% [30]. Boussaid *et al.*, (2018) reported the moisture content of six Tunisian honey samples from various floral origins ranges between 17.27-19.80 % [10]. Lullah-Deh *et al.*, (2018) studied the moisture content of seven honey samples from Mambilla Plateau, Nigeria ranges between 16.4 to 34.0% [31]. Trstenjak *et al.*, (2017) studied the moisture content of 200 Acacia honey samples obtained from different regions of Croatia varied from 16.78-17.01% [42]. Silva *et al.*, (2017) reported the moisture content of Portuguese honey from Castelo Branco region in the range of 5.53 to 24.20% [32]. Kaur *et al.*, (2016) studied the moisture content of honey samples using GIS technique in selected states of Northern India ranged from 18.0 to 24.50% [43]. Sohaimy *et al.*, (2015) studied the moisture content of Egyptian, Kashmiri, Yemeni and Saudi honey ranges between 14.73- 18.32% [34]. Ndife *et al.*, (2014) reported the moisture content of Nigerian honey sourced from different floral locations ranges from 15.69 – 18.41% [33]. Liberato *et al.*, (2013) reported the moisture content of 22 honey samples from Ceara state, Northeastern Brazil ranges between 13.63 to 20.80% [36].

The mean values of electrical conductivity in blended honey samples obtained from three different locations of Kannad taluka were within the range of 0.07 to 0.116 mS/cm. EC is one of the most considerable factors for determining the physical characteristics of honey [44]. It is the indication of ionizable acids and compounds in an aqueous solution and is a good criterion used for the identification of honey quality and purity [45].

Large amount of literature reported the different values of electrical conductivity. [46] Iftikhar *et al.*, (2014) studied the electrical conductivity of local and imported brands of honey

samples available in the Rawalpindi and Islamabad markets in Pakistan ranged from 0.08 to 0.80mS/cm.

Sajid *et al.*, (2020) comparatively studied electrical conductivity of fresh and branded honeys from Pakistan in the range of 0.11 to 0.20 mS/cm in fresh honey and 0.17 to 0.23 mS/cm in branded honeys [28]. Goncalves *et al.*, (2018) determined the electrical conductivity of selected Portuguese commercial monofloral honey samples ranges from 0.21-0.60mS/cm [29]. Kumar *et al.*, (2018) reported the electrical conductivity of 100 Indian honey samples in range 0.28-1.00mS/cm [30]. Boussaid *et al.*, (2018) reported the electrical conductivity of six Tunisian honey samples from various floral origins ranges between 0.39-0.89 mS/cm [10]. Lullah-Deh *et al.*, (2018) studied the electrical conductivity of seven honey samples from Mambilla Plateau, Nigeria in the range of 7.6 to 12.4 μ S/m [31]. Silva *et al.*, (2017) reported the electrical conductivity value of Portuguese honey from Castelo Branco region in the range of 130.2 to 667.4 μ S/cm [32]. Trstenjak *et al.*, (2017) determined the electrical conductivity of 200 Acacia honey samples obtained from different regions of Croatia ranges between 0.15 0.18mS/cm [42]. Sohaimy *et al.*, (2015) studied the electrical conductivity of Egyptian, Kashmiri, Yemeni and Saudi honey ranges between 0.53-4.18 mS/cm [34]. Buba *et al.*, (2013) reported the electrical conductivity of honey samples from North-East Nigeria ranged from 0.05-0.41mS/cm [35].

The mean values of reducing sugar in blended honey samples obtained from three different locations of Kannad taluka were within the range of 59.93 to 65.74%. Honey is a mixture of chiefly two reducing sugars namely glucose and fructose, giving it similar properties to invert syrup. This gives it the ability to remain liquid for long periods of time [47]. Sugars are the main constituents of honey comprising about 95% of honeys dry weight [48]. Reducing and non-reducing sugars together account for 85-95% of honey's carbohydrate and their amount depends on the source of nectar [49].

Goncalves *et al.*, (2018) determined the concentration of reducing sugars in selected Portuguese commercial monofloral honey samples ranges between 62.4-71.4g/100g [29]. Kumar *et al.*, (2018) determined the concentration of total reducing sugars in 100 Indian honey samples ranges between 64.91-71.39% [30]. Aljohar *et al.*, (2018) reported the concentration of total reducing sugars in honey samples available in the Saudi market ranges from 39.60-79.13% [50]. Trstenjak *et al.*, (2017) determined the concentration of total reducing sugars of 200 Acacia honey samples obtained from different regions of Croatia ranges between 68.46-70.62g/100g [42]. Kaur *et al.*, (2016) studied the total reducing sugars of honey samples using GIS technique in selected states of Northern India ranged from 65.58 to 78.51% [43]. Sohaimy *et al.*, (2015) reported the concentration of total reducing sugars in Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 15.11- 72.36% [34]. Iftikhar *et al.*, (2014) studied the total sugar content of local and imported brands of honey samples available in the Rawalpindi and Islamabad markets Pakistan ranged from 75.0 to 83.0% [46]. Alemu *et al.*, (2013) determined the reducing sugar of honey produced in Sekota district of Northern Ethiopia ranges between 63.4 to 71.7% [47].

The mean concentration of glucose in blended honey samples obtained from three different locations of Kannad taluka were within the range of 22.28 to 24.76%. Kumar *et al.*, (2018) determined the concentration of glucose in 100 Indian honey samples ranges between 26.13-46.94% [30]. Boussaid *et al.*,

(2018) reported the of glucose concentration in six Tunisian honey samples from various floral origins ranges between 31.07-36.58% [10]. Aljohar *et al.*, (2018) reported the concentration of glucose in honey samples available in the Saudi market ranges from 16.26-42.84% [50]. Sohaimy *et al.*, (2015) reported the concentration of glucose in Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 10.63-26.54% [34]. Buba *et al.*, (2013) studied the glucose content of honey samples from North- East Nigeria ranges from 27.25 to 39.56% [35].

The mean concentration of fructose in blended honey samples obtained from three different locations of Kannad taluka were within the range of 35.17 to 43.46 %. Kumar *et al.*, (2018) determined the concentration of fructose in 100 Indian honey samples ranges between 39.46-21.82% [30]. Boussaid *et al.*, (2018) reported the concentration of fructose in six Tunisian honey samples from various floral origins ranges between 35.78-37.84% [10]. Aljohar *et al.*, (2018) reported the fructose concentration in honey samples available in the Saudi market ranges from 2.63-39.14% [50]. Sohaimy *et al.*, (2015) reported the fructose concentration in Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 4.48- 50.78% [34]. Buba *et al.*, (2013) studied the fructose content of honey samples from North- East Nigeria ranges from 37.68 to 40.31% [35].

The mean F/G ratios in blended honey samples obtained from three different locations of Kannad taluka were within the range of 1.42 to 1.95. Boussaid *et al.*, (2018) reported the fructose glucose ratio of six Tunisian honey samples from various floral origins ranges between 1.03-1.17 [10]. Aljohar *et al.*, (2018) studied the fructose/glucose ratio of honey samples available in the Saudi market ranges between 0.13-1.63 [50]. Kaur *et al.*, (2016) studied the F/G ratio of honey samples using GIS technique in selected states of Northern India ranged from 0.92 to 1.18 [43]. Sohaimy *et al.*, (2015) reported the fructose/glucose ratio of Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 0.42-2.35 [34]. Buba *et al.*, (2013) studied the fructose/glucose ratio of honey samples from North- East Nigeria ranges from 1.00 to 1.45 Fructose/glucose ratio indicates the ability of honey to crystallize [35]. White and Doner (1980) stated that even though honey has less glucose than fructose, it is the glucose that crystallizes when honey granulates because it is less soluble in water than fructose [51]. When the fructose/glucose ratio is high, honey remains liquid. Honey crystallization is slower when the fructose/glucose ratio is more than 1.3 and it is faster when the ratio is below 1.0 [52].

Hydroxymethylfurfural (HMF) content of honey is the important parameter 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. [53]. The amount of HMF in honey is one of the important indicators of honey quality. In fresh honey, HMF is present only in trace amounts and its concentration increases with storage and prolonged heating of honey [48]. The mean concentrations of HMF in blended honey samples obtained from three different locations of Kannad taluka were within the range of 9.28 to 20.90 mg/kg. Goncalves *et al.*, (2018) determined the HMF content of selected Portuguese commercial monofloral honey samples ranges between 7.4-28.4mg/kg [29]. Buba *et al.*, (2013) reported the HMF content of honey samples from North-East Nigeria ranges between

5.99-17.22mg/kg^[35]. Kumar *et al.*, (2018) determined the HMF content of 100 Indian honey samples ranges between 3.65-23.16mg/kg^[30]. Boussaid *et al.*, (2018) reported the concentration of hydroxymethylfurfural of six Tunisian honey samples from various floral origins ranges between 12.07-27.43mg/kg^[10]. Trstenjak *et al.*, (2017) reported the HMF content of 200 Acacia honey samples obtained from different regions of Croatia ranges between 3.54-5.92 mg/kg^[42]. Kaur *et al.*, (2016) studied the HMF content of honey samples using GIS technique in selected states of Northern India ranged from 0.24 to 58.00 mg/kg^[43]. Iftikhar *et al.*, (2014) studied the HMF content of local and imported brands of honey samples available in the Rawalpindi and Islamabad markets of Pakistan ranged from 15 to 95 mg/kg^[46].

The mean sucrose content in blended honey samples obtained from three different locations of Kannad taluka was within the range of 1.083 to 1.634 g/100g. Goncalves *et al.*, (2018) reported the sucrose content of selected Portuguese commercial monofloral honey samples ranges between 0.6-9.4g/100g^[29]. Kumar *et al.*, (2018) reported the sucrose content of 100 Indian honey samples ranges between 0.74-3.95%^[30]. Boussaid *et al.*, (2018) determined the sucrose content of six Tunisian honey samples from various floral origins ranges between 0.20-4.60%^[10]. Aljohar *et al.*, (2018) studied the sucrose content of honey samples available in the Saudi market ranges between 0.00-24.6%^[50]. Kaur *et al.*, (2016) studied the sucrose content of honey samples using GIS technique in selected states of Northern India ranged from 0.41 to 3.10g/100g^[43]. Sohaimy *et al.*, (2015) determined the sucrose content of Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 1.34- 3.59g/100g^[34]. Iftikhar *et al.*, (2014) studied the sucrose content of local and imported brands of honey samples available in the Rawalpindi and Islamabad markets Pakistan ranged from 7.60 to 8.70 %^[46]. Buba *et al.*, (2013) studied the sucrose content of honey samples from North- East Nigeria ranges from 0.53 to 3.29gm/100gm^[35].

The mean concentrations of Proline in blended honey samples obtained from three different locations of Kannad taluka were within the range of 752.80 to 1039.97 mg/kg. Sajid *et al.*, (2020) comparatively studied proline content in fresh and branded honey from Pakistan in the range of 287.60 to 511.1mg/kg in fresh honey and 103.66 to 329.66mg/kg in branded honeys^[28]. Goncalves *et al.*, (2018) determined the concentration of proline in selected Portuguese commercial monofloral honey samples ranges between 412.3-566.6mg/kg^[29]. Boussaid *et al.*, (2018) reported the proline content of six Tunisian honey samples from various floral origins ranges between 39.62-102.22mg/kg^[10]. Cherian *et al.*, (2011) studied the proline content of honey Produced by *Apis cerana indica* of Nagpur, Maharashtra (India) ranges between 131.1 to 423.4 mg/kg^[54]. Proline is one of the important amino acid found in honey. Proline was a criterion of honey ripeness and sugar adulteration^[55].

The mean concentration of protein in blended honey samples obtained from three different locations of Kannad taluka were within the range of 1.35 to 2.02 g/kg. Goncalves *et al.*, (2018) reported the protein content of selected Portuguese commercial monofloral honey samples ranges between 0.16-0.57g/100g^[29]. Kumar *et al.*, (2018) reported the protein content of 100 Indian honey samples ranges between 0.16-0.70%^[30]. Boussaid *et al.*, (2018) determined the protein content of six Tunisian honey samples from various floral origins ranges between 0.13-0.16%^[10]. Sohaimy *et al.*, (2015)

determined the protein content of Egyptian, Kashmiri, Yemeni and Saudi honeys ranges between 1.69- 4.67mg/g^[34]. Ndife *et al.*, (2014) reported the protein content of Nigerian honey sourced from different floral locations ranges from 0.90 – 1.15%^[33]. Liberato *et al.*, (2013) reported the protein content of 22 honey samples from Ceara state, Northeastern Brazil ranges between 178 to 1121µg/gm^[36]. Buba *et al.*, (2013) studied the protein content of honey samples from North- East Nigeria ranges from 0.35 to 1.08gm/100gm^[35]. Protein content in honey samples is reported to consist of mostly enzymes^[56]. Cimpoiu *et al.*, (2013) reported the total protein content of some Romanian honey ranges between 173 to 763µg/gm^[57]. Khalil *et al.*, (2001) reported the protein content of different brands of unifloral honey available in the Northern region of Bangladesh in the range of 0.65 to 0.744%^[58].

The mean values of lipid content in blended honey samples obtained from three different locations of Kannad taluka were within the range of 0.27 to 0.31 g/100g. Ndife *et al.*, (2014) reported the lipid content of Nigerian honey sourced from different floral locations ranges from 0.12 – 0.21%^[33]. Khalil *et al.*, (2001) reported the lipid content of different brands of unifloral honey available in the Northern region of Bangladesh ranges between 0.134 to 0.146 gm/100gram^[58]. Buba *et al.*, (2013) reported the lipid content in honey samples from North- East Nigeria ranges from 0.10-0.50gm/100gm^[35]. Reports indicating that honey contains little or no fat are available in the literature^[59, 60], but the presence of free fatty acids like palmitic, oleic and linolenic acids have been reported in white clover honey, thus indicating that honey consist of a very little amount of lipid and therefore not considered a good source of lipid^[35].

The mean concentrations of Vitamin C in blended honey samples obtained from three different locations of Kannad taluka were within the range of 626.33 to 949.55 mg/kg. Rahman *et al.*, (2014) found the values of vitamin C in the range of 100 to 1770mg/kg in Pakistan honey^[61]. Buba *et al.*, (2013) determined the concentration of Vitamin C in honey samples from North- East Nigeria ranges from 13.86 to 27.32mg/100gm^[35]. Kesic *et al.*, (2009) reported the concentration of vitamin C in the range of 37.22 to 378.30 mg/100gm in honey from different locations of Bosnia Herzegovina^[62]. Matei *et al.*, (2004) determined the vitamin C content and some essential trace elements (Ni, Mn, Fe, Cr) in bee products and the value of vitamin C ranges between 2.26 to 3.64mg/gm^[63]. Khalil *et al.*, (2001) reported the vitamin C content of different brands of unifloral honey available in the Northern region of Bangladesh ranges between 4.2 to 6.25mg/100gm^[58].

Honey consists of ascorbic acid because most flowers on which the bees forage contain this vitamin which serves as an antioxidant in addition to many other functions. Even, it has been shown that antioxidant activity of honey, which depends on its botanical origin, is related to its vitamin C contents; i.e., the content of vitamin C has a significant impact on the total antioxidant activity of honey^[62].

4. Conclusion

Overall results of physiochemical parameters indicate that the nutritional quality of honey was different from species to species and from location to location. It might be due their foraging sources. The honey obtained from agricultural and forest areas has highest nutritional quality than honey obtained from road side area. The average value of the

physicochemical parameters found in the honey samples showed that honey harvested from the studied area is safe for human consumption according to Codex Alimentarius

standards [64, 65, 66] and for the commercialization of beekeeping practices to improve quality in the future this study utmost needed.

Table 1: Physical properties of blended raw honey harvested from three different locations of Kannad taluka of Aurangabad district.

Collection Site	pH	Electrical Conductivity (mS/cm)	Moisture Content (%)
Agricultural Area	4.2±0.15	0.093±0.001	17.33±0.94
Forest Area	3.8±0.12	0.07±0.002	15.96±0.92
Road Side Area	5.3±0.22	0.116±0.005	18.23±0.88
Standards of Codex, 1993 and 2019.	3.4-6.1	0.8 mS/cm	22%

Table 2: Biochemical properties of blended raw honey harvested from three different locations of Kannad taluka of Aurangabad district.

Site of Collection & Parameters	Glucose %	Fructose %	Total reducing sugar %	Sucrose gm/100gm	G/F ratio	HMF Content mg/kg	Proline Conc.mg/kg	Protein gm/kg	Lipid gm/100gm	Conc. of Vitamin C mg/kg.
Agricultural Area	23.63±0.89	39.09	62.72±2.14	1.31±0.38	1.65	14.52±0.72	990±24.15	2.02±0.2	0.30±0.07	828.43±17.88
Forest Area	22.28±0.89	43.46	65.74±1.4	1.63±0.22	1.95	9.28±0.71	1039.97±28.9	2.01±0.2	0.27±0.08	949.55±31.44
Road Side Area	24.76±0.51	35.17	59.93±1.73	1.08±0.31	1.42	20.90±0.8	752.80±16.4	1.35±0.1	0.31±0.08	626.33±13.75
Standards of Codex, 1998 and 2019.	23-32%	31.2-42.4%	> 60%	< 5 gm/100gm	> 0.95	< 40 mg/kg	> 180 mg/kg	> 0.1 gm/kg	0.10-0.50 gm/100gm	No fixed limits

± indicates standard deviation.

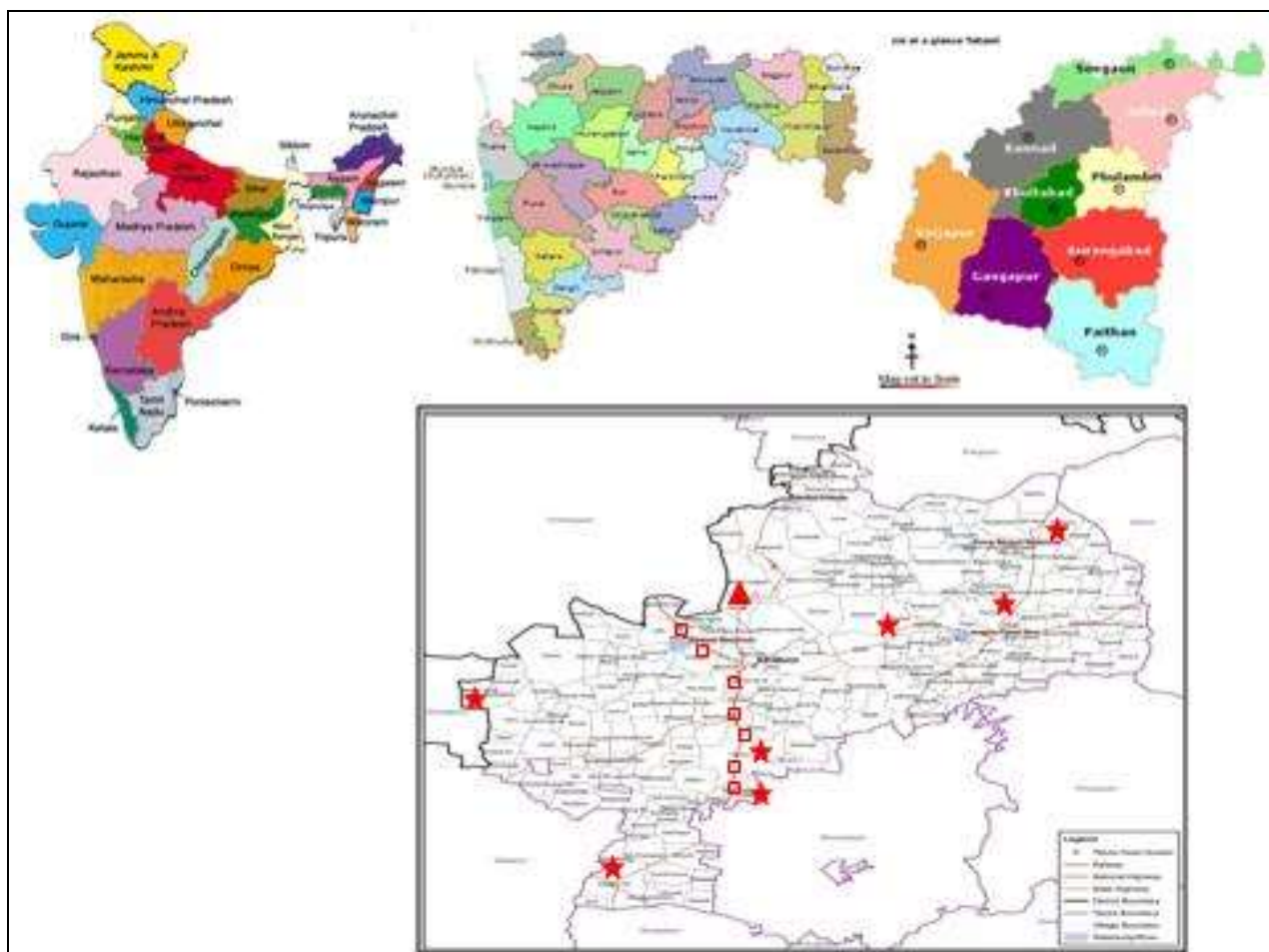


Fig 1: Location map

5. Acknowledgements

Authors are thankful to Head, Dept. of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing the necessary laboratory facilities during the present work and university authorities for granting Golden Jubilee University Scholarship.

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