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Physicochemical and biochemical analysis of three Indian unprocessed unifloral honey varieties

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Abstract

Unifloral honey seeks premium price in the market due to its distinct color, taste, fragrance and its characteristics. Each flower puts its unique characteristic in a unifloral honey. Even the health and medicinal value of unifloral honey depends on the type of flower. The unifloral honey samples were analyzed for their physico-chemical and biochemical properties. The moisture content values varied from 16.54 ±0.15 % to 17.45 ± 1.04 %. eucalyptus honey (EH) showed the highest moisture content. Total soluble solids (TSS) in neem honey (NH) was found to be the highest (81.73 ± 0.14), compared to tulsi honey (TH) (81.50 ± 0.54) and EH (80.01 ± 1.10). Specific gravity of all three honey samples were 1.4131 ±0.00, 1.4053 ±0.00, and 1.4246 ±0.00 for NH, EH and TH respectively. The ash content was 0.1±0.02 %, 0.18±0.03 %, and 0.21±0.02 % in the NH, TH, and EH samples respectively. The pH values were 3.49 ± 0.04 for NH, 3.33 ± 0.02 for EH and 3.14 ± 0.01 for TH. The total phenolic content value was found in the order of NH > TH > EH. The values were 27038.9 ± 0.07, 13071.61 ± 0.07, 6765.15 ± 0.03 (mg GAE / Kg of honey) respectively. The L* values were 46.63 ± 1.63 for NH, 56.81 ± 1.28 for EH, and 51.91 ± 1.37 for TH. The a* values were 6.26 ± 0.07, 5.45 ± 0.19, and 3.22 ± 0.06 for NH, EH and TH respectively. The b* values were 37.73 ± 1.09 for NH, 31.45 ± 0.63 for EH, and 32.84 ± 1.14 for TH. The antiradical scavenging activity values for TH (80.36±0.32 %), NH (77.23 ±0.77 %) and EH (73.09 ± 1.31 %).

Keywords: India, unifloral honey, physico-chemical activity, total soluble solids

Introduction

Honey is a worldwide natural sweetener which has loads of health benefits. Climatic conditions, plant sources, environmental conditions are the some factors which are responsible for the composition of the honey. In recent years the knowledge about the unifloral honey has cropped up among the bee keepers and consumers. This lifted up the honey production in India. Honey with specific characteristics can be produced by distinct floral species, in terms of flavor, color and unique chemical composition. Each type of unifloral honey has its own health benefits. Honeybees collect nectar from various flowers to make honey. Bees gather 50-70 mg of nectar, by visiting nearly 100-1500 flowers, which is further processed to make honey.

Due to botanical and geographical origin the composition of the honey is varied. Honey contains about 80% of sugars (Glucose, fructose, maltose, raffinose, sucrose, trehalose, isomaltose, erlose, turanose), and the remaining 20% is organic acids (Lactic, butanoic, succinic, gluconic, formic, citric, acetic, malic, pyroglutamic, and a large number of aromatic acids), water, amino acids (serine, proline, methionine, histidine, isoleucine, arginine, valine, ornithine, asparagine, tyrosine, lysine, leucine), group B Vitamins (niacin, folic acid, pyridoxine, pantothenic acid, riboflavin and Vitamin C), enzymes (diastase, glucose oxidase, invertase, catalase) and minerals (iron, zinc, calcium, phosphorous, manganese, magnesium and potassium) (Scripcă et al., 2019). Color is one of the physical properties of honey that consumers notice the most. It is used as a criterion for honey acceptance. Furthermore, the value of honey is related to its colour. Depending on the botanical origin, age, and storage conditions the color of the honey varies. To determine the botanical origin and quality of honey, a standard method of colour determination is required. The Commission Internationale d'Eclairage (CIE) L*a*b* method is majorly used to describe honey color. Water is the second most important component of honey, after sugar, moisture, which is primarily related to a product's aw, which has an impact on food stability (Belay et al., 2017).

It is essential to note that honey is rarely sold in its raw form; rather, it is processed. This is primarily due to consumer demand for a fluid, non-crystallized product, and while freshly harvested raw honey is in a liquid state, it crystallizes at varying rates. Numerous factors influence crystallization, including origin (botanical and geographical), sugar content, temperature, and moisture content, (Mar Cavia et al., 2009). During processing, honey's antioxidant activity, colour darkening, and rheological properties may change, all of which have a negative impact on its quality and mask its originality. Thermal treatment in industrial manufacturing of honey includes two steps. Liquefaction (approx. 55oC) ensures that the honey is sufficiently liquid to handle; and pasteurization (approx. 80oC) destroys yeast that may cause unwanted

fermentation during the product's shelf life (Escriche et al., 2014).

Already many researches were carried out in past for the characterization of multifloral and unifloral honey in worldwide in Nepal, India, Australia etc., (Saravana Kumar & Mandal, 2009), (Tanleque-Alberto et al., 2020), (Basavarajappa et al., 2011), (Oroian, 2012). This study is aimed to analyze the physico-chemical and the biochemical properties of unprocessed unifloral honey varieties which are specific to Indian origin. The Honey samples taken for this study are collected from the unique plant sources of India like Neem, Eucalyptus and Tulsi.

Materials and Methods

Sample Collection and Preparation

Three different unprocessed, fresh unifloral honey samples were collected directly from the apiarists form different places of India. The collection area and the collection period of honey samples were mentioned in Table-1. The samples were stored at 28oC until analysis. All the analyses were performed within 30 days of sample collection. If the samples were granulated the closed container was placed in water bath without submerging and they were heat at 50-60oC until liquefied (Food Safety & Standards Authority of India, 2015) before analysis. The major plant sources of the three honey samples were Neem, Eucalyptus, and Tulsi. They were marked as NH (neem honey), EH (eucalyptus honey), and TH (Tulsi honey). All the three samples were collected from *Apis mellifera* (western honey bee) bee hives.

Physico-chemical parameters

The honey samples were analyzed for their ash content, total soluble solids (TSS),specific gravity, and moisture content (Food Safety & Standards Authority of India, 2015). ATAGO refractometer (Model -RX- 700α, Atago Co., Ltd., Itabashi – Ku, Tokyo) was used for the moisture content analysis. Pyknometer (25ml) was used for specific gravity analysis. For TSS and moisture content all the measurements were carried at 20oC and the results were expressed in ºBrix and percentage respectively. All the analyses were carried out in triplicates.

Water Activity

The water activity (aw) of honey samples was determined by using water activity meter (Model 4TE Aqua Lab Dew Point water activity meter). About 2g of the sample was taken for the determination of water activity. The water activity of the sample was measured based on its equilibrium relative humidity (ERH). The relationship between ERH and aw is aw = ERH (in percentage) / 100 (Saxena et al., 2010)

pH

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

Color Analysis

Color parameters of the honey samples were measured by using Hunter Lab Color Flex EZ, bench top color spectrophotometer (Hunter Laboratory Associates, Inc., Reston, Virginia, USA). The principle of Hunter Color lab is focusing on light and measured energy reflected from the sample across the entire visible spectrum. (Ferrari et al., 2010, Janghu et al., 2018).

Biochemical properties

Total Phenolic Content

The total phenolic content was determined by the Folin – Ciocalteau's method (Saxena et al., 2010) (Pontis et al., 2014) with few modifications. About 0.5 g of each honey samples was dissolved in 5 ml of distilled water. From the above solution 0.5ml of aliquot was fixed with 4.5ml of deionized water followed by the addition of 0.5ml 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 725nm 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 0.5ml of aliquots was taken and mixed with 1ml of 1mM DPPH prepared in ethanol. The final volume was made upto 5ml 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 517nm. Antiradical scavenging activity was calculated using the following formula:

Antiradical activity (%) = (Absorbance of control-absorbance of sample / absorbance of control) X 100…. 1

Statistical analysis

Each honey samples was analyzed 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 ash content, moisture, and specific gravity of all three unifloral honey samples (NH, EH and TH) collected from different plant sources are presented in Table 2. Sample EH showed significantly higher ($p < 0.05$) moisture content, whereas NH sample showed lowest moisture content. The moisture content values varied from 16.54 ±0.15 % to 17.45 ± 1.04 %. Moisture content of unifloral honey may vary on the composition due to variable floral sources, temperature, humidity conditions, stage of harvesting etc. Stability of honey is directly related to the moisture content. Honey with higher moisture content has more water activity and it is more susceptible to microbial spoilage than honey with a lower moisture content (Janghu et al., 2018). As a result of the aforesaid findings, it can be stated that NH may be less resistant to microorganisms/microbial deterioration than the

other two samples. Total soluble solids (TSS) were found to be the highest in NH and the value was81.73 ± 0.14. Next to NH, TH had the 81.50 ± 0.54 of TSS and the least TSS was found in EH with 80.01 ± 1.10. Specific gravity of all three samples was 1.4131 ±0.00, 1.4053 ±0.00, and 1.4246 ±0.00 for NH, EH and TH respectively. The ash concentration of all three honey samples differed significantly ($p < 0.05$), with the ash content ranges of 0.1±0.02 %, 0.18±0.03 %, and 0.21±0.02 % in the NH, TH, and EH samples respectively (Table 2). Geographical, floral, and meteorological variations may all contribute the variations in ash content. Our findings were online 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 of unifloral honey samples were 3.49 ± 0.04 for NH, 3.33 ± 0.02 for EH and 3.14 ± 0.01 for TH respectively (Table-2).

Table -1: Plant source and collection area of unifloral honey samples

Sl.no	Plant Source	Honey Type	Scientific Name of the Plant, Collection area & Collection period
1.		Neem Honey (NH) 	*Azadirachta indica* Karnataka, India March & April, 2020
2.		Eucalyptus Honey (EH) 	*Eucalyptus globulus* Haryana, India March & April, 2020
3.		Tulsi Honey (TH) 	*Ocimum tenuiflorum* Kashmir, India August to October, 2020

Water activity

The water activity was noted down as 0.5989 ± 0.01 for NH, 0.6466 ± 0.01 for EH, and 0.6317 ± 0.02 for TH (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 (Kňazovická et al., 2015).

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 (Scripcă et al., 2019). The lightness of honey was represented by the L* parameter, and the values were 46.63 ± 1.63 for NH, 56.81 ± 1.28 for EH, and 51.91 ± 1.37 for TH. The green compound (negative a* values) was represented by a* parameter. It was not found in any of the samples. The red parameter (positive a* values) was discovered in all the three samples. The values were 6.26 ± 0.07, 5.45 ± 0.19, and 3.22 ± 0.06 for NH, EH and TH respectively. The yellow parameter (positive b* values) was discovered in all samples and the values were 37.73 ± 1.09 for NH, 31.45 ± 0.63 for EH, and 32.84 ± 1.14 for TH (Table-2). Our values were in accordance with the previous research works done with unprocessed unifloral honey samples (Janghu et al., 2018).

Biochemical Properties

Total Phenolic Content

The total phenolic content of unifloral honey was in the order of NH > TH > EH and the values were 27038.9 ± 0.07 (mg GAE / Kg of Honey) > 13071.61 ± 0.07 (mg GAE / Kg of Honey) > 6765.15 ± 0.03 (mg GAE / Kg of Honey) (Fig-

1). Dark honeys have higher total phenol content than light honeys from different geographical sources (Gül & 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 higher phenolic content of the neem honey might be due to the presence of flavonoids and antioxidants naturally.

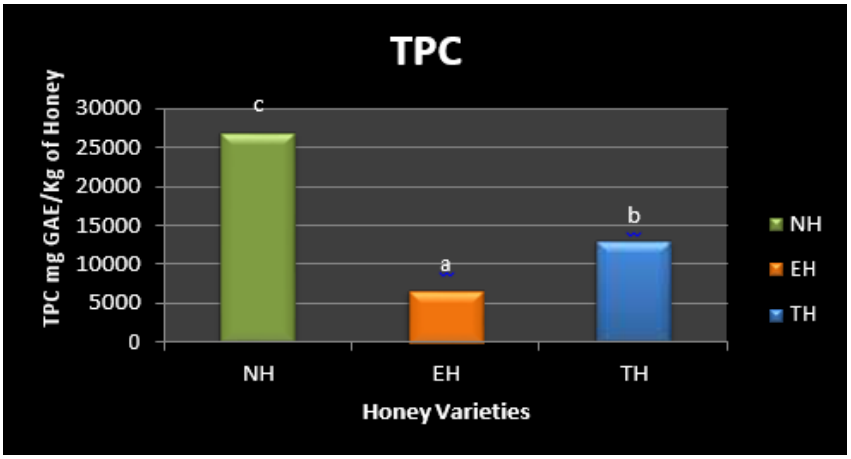

Fig 1: Total Phenolic Content of three unprocessed unifloral honey samples in mg GAE/Kg of honey

DPPH antiradical scavenging activity

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 higher in TH (80.36±0.32 %), than the NH (77.23 ±0.77 %) and EH (73.09 ± 1.31 %). Due to the presence of antioxidants naturally from the plants such as Tulsi, eucalyptus and neem the all three samples showed above 70% of the antiradical scavenging activity.

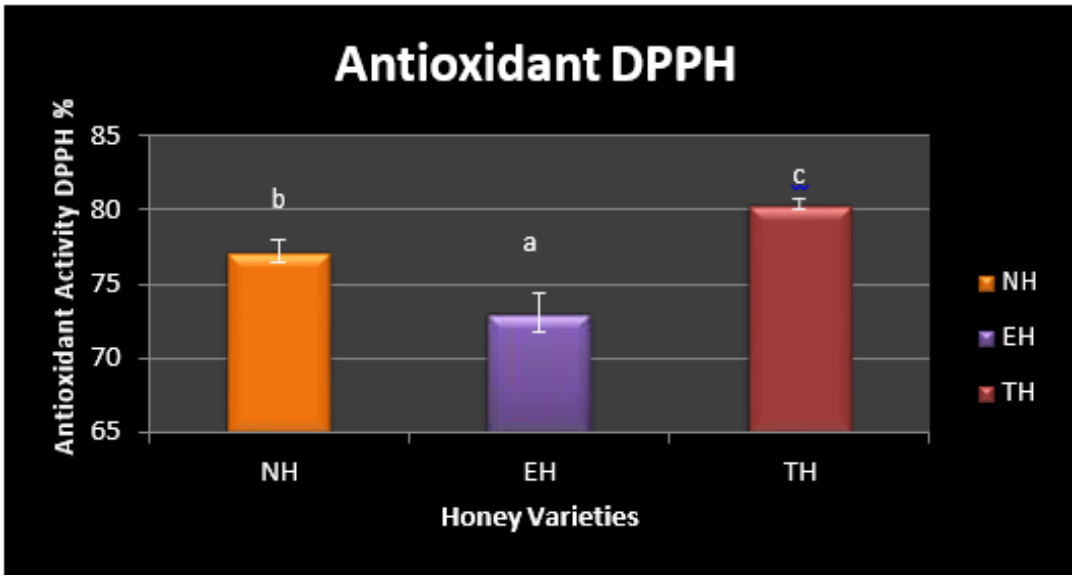

Fig 2: Antiradical scavenging activity of three unprocessed unifloral honey samples

Conclusion

The present study established the correlation between the physico-chemical parameters of honey such as moisture content, water activity, pH, TSS, ash content etc., Future studies to identify the 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). Also, future studies are needed about the biological activities of individual honey samples reveals the benefits of unifloral honey in daily usage will enlighten the knowledge about the benefits of unifloral honey varieties among the researchers and consumers.

Table 2: Physico-chemical properties of unprocessed unifloral honey varieties

	Moisture Content (%)	Water Activity	Ash Content (%)	TSS (obrix)	Color			pH	Specific Gravity
					L	a	b		
NH	16.54± 0.15a	0.5989± 0.01a	0.1± 0.02a	81.73±0.14b	46.00± 1.63c	6.25± 0.07a	37.73 ± 1.09a	3.49 ± 0.04c	1.4131±0.0a
EH	17.45± 1.04a	0.6466± 0.01b	0.21± 0.02b	80.01± 1.10a	56.81± 1.28a	5.45± 0.19b	31.45 ± 0.63c	3.33 ± 0.02b	1.4053±0.0a
TH	16.77± 0.56a	0.6317± 0.02b	0.18± 0.03b	81.50± 0.54b	51.91± 1.37b	3.22± 0.06c	32.84 ± 1.14b	3.14 ± 0.01a	1.4246±0.0a

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

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