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Estimation of physicochemical characteristics of honey from various botanical sources

Ajay Kumar[1], Rakesh Kumar[2], Neelam Pathak[3], Ramesh Pratap Singh[4], H C Varma[5] R. K. Dohary[6] and Shambhoo Prasad[7]*

[1]Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.
[2]Department of Chemistry, JJT University, Jhunjhunu, Rajasthan, India.
[3]Department of Biochemistry, Ram Manohar Lohia, Avadh University, Ayodhya, Uttar Pradesh, India.
[4]Department of Biochemistry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.
[5]Department of Animal Genetics and Extension Education, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India
[6]Department of Agricultural Extension Education, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.
[7]Department of Molecular Biology and Biotechnology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India
*Corresponding author: *shambhoobiotech@nduat.org*

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Abstract

Honey is a natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew. Thus, the present study was done to assess to study the regional variations in the physicochemical parameters and bioactive compounds content of Mustard (*Brassica sp.*), Lychee (*Litchi chenensis*) and Eucalyptus (*Eucalyptus sp.*) honeys from Bikapur, Rudauli, Sohawal and Sadar tehsils of the Ayodhya district of Uttar Pradesh (UP), India. Total 36 samples (Mustard, Lychee and Eucalyptus origin, 3 samples each from each tehsil) were studied. Among all studied samples the moisture content was ranged between 15.9 to 20.7 %; the highest value being observed in litchi honey. The pH and free acidity was ranged between 3.3 to 5.2 and 12.50 to 32.50 meq/kg respectively; highest pH values were recorded in Eucalyptus honey, whereas highest free acidity was reported higher in Brassica honey. The total sugar, reducing sugar and non-reducing sugar was ranged between 71.97 to 81.7, 59.5 to 76.84 and 4.15 to 14.30 %; the total sugar, reducing sugar and non-reducing sugar being highest in Eucalyptus, Litchi and Brassica honey respectively. Electrical conductivity of honey samples

existed between the 0.12 to 0.85 µs/cm. The mean value of electric conductivity of Eucalyptus honey was highest in Sadar tehsil (0.29±0.05 µs/cm), followed by Sohawal tehsil (0.25±0.04 µs/cm), Rudauli tehsil (0.24±0.02 µs/cm) and least in Bikapur tehsil ((0.16±0.03 µs/cm). The HMF content of honey samples was differed from 4.0 to 10.0. The overall mean value of Pfund scale of Eucalyptus, Brassica and litchi honeys was 46.25±1.10, 42.83±0.99 and 67.83±1.84 mm, respectively. Thus, the variation in physicochemical parameters of honey were related its botanical floral source and geographical area from where its derived.

Introduction

Honey has long been regarded as one of the world's most prized and highly regarded natural items. Nectar from various flowers and tree nectars is gathered by honey bees and turned into nectar honey (Codex Alimentations, 2001). Honey's chemical makeup is largely determined by the climate and location in which it is gathered, as well as the variety of plants used in its production (El-Metwally 2015). At least 200 chemicals are found in honey, the majority of which are carbohydrates and water. Aside from these components, it also includes trace amounts of various vitamins and organic acids, as well as trace amounts of various minerals, free amino acids, enzymes, and flavonoids and other phytochemicals (Abselami, 2018). Some of the compounds in honey have been shown to have antioxidant and antibacterial properties as well as inflammatory and proliferation inhibiting as well as cancer-fighting and antimetastatic properties (Adgaba *et al*., 2021). The wound-healing properties of honey are also worth noting.

Honey contains a significant amount of antioxidant flavonoids and polyphenols. Aside from its nutritional value, honey is utilized in traditional medicine for a variety of medical issues, from wound healing to the treatment of tumors, in addition to its nutritional value. Many kinds of antioxidants found in honey have been shown to be beneficial in the treatment of a wide range of diseases, including cancer. Honey is a notable candy substance obtained from honeycomb. According to Codex Alimentarius "Honey is an herbal sweet substance produced with the aid of the honey bees from the nectar of blossoms or from the secretion of living elements of flora or excretion of plant-sucking bugs at the components of plants, which honey bees accumulate, transform and integrate with precise materials in their own and save within the honey comb to ripen and mature" (Encyclopaedia Britannica,1991). From historical time use of honey for numerous functions, viz. as meals, remedy and rituals, is a conventional exercise. The importance of honey has been referred to inside the historical Indian Vedas.

Honey is produced by means of bees however it’s not just these bugs that benefit from it. Most people consider this sweet-tasting ingredient to be a pantry staple since it can be used in desserts and baked goods, as well as more savory foods. If you're in the mood for something sweet, try it on a piece of bread or in your morning cup of tea. Honey is likewise concept to have some health blessings. it may be combined with lemon and warm water as an example, to create a soothing liquid that’s able to calming a sore throat or cough. So, the component is fairly flexible as well as useful for our fitness. The honey bees collect a sugary substance referred to as nectar from a spread of flora. They suck this nectar up with their tongues and then save in their honey stomach (that is break away their normal stomach). Once their honey stomach is full, those bees go back to the hive and, the usage of their mouths, they bypass their series directly to employee bees. These bees chew the nectar for a while until it subsequently becomes honey.

A number of the most extensively available honeys include clover and orange blossom. a number of the greater uncommon sorts, which additionally have a tendency to have a stronger taste, consist of Manuka, eucalyptus and Scottish heather. Honey can be used as a herbal sweetener in various dishes along with muesli and also can be used in preference to sugar in sweet cakes (Eleazu *et al.,* 2013). This adds some of dietary fitness advantages for the breathing organs and the digestive device. Precious strains of enzymes, vitamins and minerals are also found in honey in small quantities (Erturk *et al.,* 2019). The nutritional value of 50g of honey is as follows: 152 Kcal energies, 42.2g carbohydrates, 0g fat and 0.15g protein (Gaur, *et al.,* 2014).

Honey is an excessive excellent carbohydrate this is without problems absorbed through the body. That's because dextrose and fructose, two sugars that are contained in honey in excessive amounts, pass immediately into the blood circulate without setting a strain at the digestive gadget (Azonwade, 2018). Coughs and colds are relieved when honey is consumed in hot water or in combination with other therapies, such as lemon. Honey is an excellent supplement to prescription medication in situations of catarrh or bronchial tube or lungs disease (Belay *et al*., 2013). If you have arthritis or gout, honey applied topically with a warm substance may provide exceptional relief. In addition, honey may be used as a salve to treat dry lips, grazes, boil or small wounds (Bogdanov, 2008). Moreover, honey has antibacterial properties. Because honey has so many advantages, it's easy to understand why it's so popular today.

Materials and methods

The present study entitled "Evaluation of Physicochemical parameters of honey obtained from different floral origins of Ayodhya district of Uttar Pradesh, India" was studied in the three tehsils namely Bikapur, Rudauli, Sohawal and AyodhyaSadar of Ayodhya district of Uttar Pradesh. The laboratory works were conducted in the Department of Agiculture Biochemistry, College of Agriculture, AcharyaNarendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, UP and Regional Food Research &Analysis Centre, Department of Horticulture and Food Processing, Lucknow, UP during the period 2020-2022.

The details of study material used, plans of experiment and methodology adopted in this study is explained herein this chapter under the following sub-headings.

Honey of Apis mellifera was collected from the four tehsils namely Bikapur, Rudauli, Sohawal and Sadar of Ayodhya district of Uttar Pradesh. Ayodhya is one of the important districts of eastern Uttar Pradesh. Ayohaya is pilgrims place of Hindus andis known as birthplace of lord Rama. River Saryu and Gomati making the borders of Ayodhya district in north and south, respectively. It is 93 meters above sea level on average (305 feet). Ayodhya (Awadhpuri) has been recognized as the first of the seven most important Hindu pilgrimage sites due to the belief that it is the birthplace of Bhagwan Shri Ram.

The moisture content was estimated by taking a measurement of 10.0 grammes of honey that had been placed on an aflat dish and baked at 105 degrees Celsius for three hours. After that, it was sealed, placed in desiccators to chill it down, and weighed. After being heated to 105 degrees Celsius in the oven for an hour, the sample was then allowed to cool before being reweighed. The procedure was carried out once per hour at predetermined time intervals until a stable weight was achieved (Kayode and Oyeyemi 2014).

$$\text{Moisture content} = \frac{(W1 - W2)}{(W1 - W0)}$$

pH value:

In a beaker with a capacity of 250 ml, ten grammes of honey and seventy-five ml of distilled water were combined. A magnetic stirrer was used to complete the mixing process. After placing the electrodes of the pH metre in the solution and recording the results, the pH was determined (A.O.A.C., 1984).

Free acidity value

Procedure:

In a 250 ml beaker, 75 ml of distilled water was mixed with 10 grammes of honey. A magnetic stirrer was used to help in the mixing process. At a rate of 5 ml per minute, the titration was continued until the pH hit 8.5 and the burette measurement was recorded. Additionally, a blank test was carried out. During the titration, sodium hydroxide is used so that the sample's free acidity was calculated (A. O. A. C., 1984). The amount of free acidity that honey has is expressed as a mg of free acids per kg of honey. The honey samples that have been dissolved in water are titrated with the use of a solution of sodium hydroxide that has a concentration of 0.1 N. The pH level is often employed as an indicator.

Electrical conductivity:

Sugars, acids, minerals, water, and a variety of minor components make up honey. Honey's electrical conductivity is regulated by the Codex Standard for Honey, which says that it shall not exceed 0.8 mS/cm (Codex Alimentarius Committee on Sugars, 2001). However, because this conductivity was determined after diluting 20 g of anhydrous honey with 100 ml of water, it is unrelated to this study. Because honey is so varied in physical features are difficult to anticipate or link to its composition. The mobility of ions is reflected by the electrical conductivity of a hydrated solution.

Hydroxy methyl furfural (HMF) content:

The absorbance A of the standard solution was measured in 1 cm quartz cells at 285 nm with water as the blank cell. From the figures found in the scientific literature for molar absorptivity, =16830, or absorptivity, at 1 percent 1cm = 133.57, one might derive the formula for calculating the concentration of the standard solutions. The concentration, expressed in mg/l, may be calculated as follows: The absorbance of the standard solution is denoted by the letter A. It is essential that the computed amount be in accordance with the requirements given by the provider. The standard needs to be kept in a nitrogen atmosphere at a temperature of 4-8 degrees Celsius. It attracts and retains a great deal of moisture.

Sugar analysis:

Carbohydrates are the primary components of a wide range of foods. Carbohydrates are found in food as polysaccharides like starch or monosaccharides and disaccharides like glucose, fructose, lactose, and sucrose, among others. Sugars, which contain all of the food's mono and disaccharides, are responsible for a variety of qualities.

Sugars are classified as reducing or non-reducing. Sugars with one or more reducing groups are referred to as reducing sugars. Except for sucrose, all mono and disaccharides are reducing sugars. Honey is a sugar solution in the form of a liquid. Honey is mostly composed of glucose and fructose. Honey's diverse qualities are solely related to its sugar profile.

Apparent reducing sugar content:

The approach works on the principle of sugar reduction to reduce Fehling's solution. Potassium sodium tartarate usually known as Rochelle salt and copper sulphate make up Fehling's solution. The reducing group in the sugar reduces the cupric hydroxide produced to cuprous oxide. Cuprous oxide is red in colour and insoluble. The end point is usually determined using methylene blue indicator.

For the estimate of reducing sugar, the following reagents were utilized. For the experiments, the regents were newly made.

Fehling's solution A:

On the electronic balance, 34.639 g of copper sulphate pentahydrate (CuSO4.5H2O) crystals were precisely weighed. The crystals of copper sulphatewere solvated in around 100 ml of distilled water in a volumetric flask of 500 ml quantity. The volume of the solution was made up after the crystals were entirely dissolved. Before usage, the fluid was double filtered with glass wool.

The copper sulphate solution was then standardised with a standard sugar solution that had already been prepared. The standardisation was carried out in order to determine the amount of invert sugar needed to reduction of copper sulphate. The titration was performed using a normal invert sugar solution. As an indication, methylene blue was utilised.

Fehling's solution B

346 g Rochelle salt (potassium sodium tartrate) and 100 g sodium hydroxide were precisely weighed and placed into a one-liter volumetric flask. The reagents were made to solvate in water according to the specifications. The volume was then made up to 1000 ml, which is known as Fehling's B solution. The Fehling's solution B was then held for a day before being used, after which it was filtered.

Invert Sugar solution:

Sucrose was weighed exactly 0.95 g and dissolved in 500 ml pure water. For the inversion, just 2 ml concentrated HCl was mixed to the sucrose solution. After boiling for thirty minutes, the solution was set aside for 24 hours.

It was neutralised with a sodium carbonate solution. Finally, the contents were transferred to a 1000 ml volumetric flask and distilled water was used to make up the volume. Standard sugar solution was utilised for all subsequent titrations. Invert sugar is present in the solution at a concentration of 0.1 g per 100 ml.

Methylene blue indicator:

The experiment was conducted with a newly made methylene blue indicator.

Procedure:

One gram of honey was obtained as a sample (H1). It was poured into a 250 ml volumetric flask that was previously empty. A hundred and fifty millilitres of water were poured to the flask, and the honey was thoroughly dissolved in it. With distilled water, the volume was increased to 250 ml after thorough mixing. This is a honey sample solution.

In a porcelain dish, five ml of each Fehling's solution A and B were obtained using two different pipettes. Filling the burette column with the sample honey solution made in the previous paragraph maintained it ready. The contents of the porcelain dish from the burette at one were instantly heated to boiling after 10-12 ml of this solution was poured. To check direct heating, asbestos gauze heater was used for heating. Methylene blue dye in three to four drops

was added to the substance in the next stage. The honey titration was preceded by keeping the solution in the porcelain dish at a high temperature. The titration reached its end point when the colour changed from blue to red (copper sulphatereduced into cuprous oxide). The honey solution volume used to complete the titration was measured and recorded. According to the formula, the proportion of reducing sugar present in the honey solution was computed.

Calculation:

$$\text{Total reducing sugar} = \frac{S \times 250 \times 100}{M \times V1}$$

Where,

V1 = Burette reading of honey solution in ml

S = Strength of the $CuSO_4$ solution

M = Weight of honey in gram

The non-reducing sugar must be turned into the reducing sugar before the total sugar as well as non-reducing sugar can be determined. An inverted sugar solution was created to perform this conversion. A 100 ml honey solution that had been prepared previously was taken. The solution was heated to boiling with 1 ml of strong hydrochloric acid. For sugar inversion, the solution was maintained overnight.

Procedure :

The inverted honey solution was then neutralised with sodium carbonate solution before being titrated with the help of Fehling's solution to verify the reducing sugar using the same process as previously described for estimating the reducing sugars. The given formula was used to calculate/find out the quantity in honey, i.e. sucrose.

Calculation :

$$\text{Total reducing sugar} = \frac{S \times 250 \times 100}{M \times V2}$$

Where

V2 = Burette reading of inverted honey solution in ml

S = Strength of the CuSO4 solution

M = Weight of honey in gram

Total reducing sugar = Total Sugar - Reducing sugar

Color analysis by Pfund scale:

Liquid honey comes in a variety of colours, ranging from nearly colourless (water) to dark amber that resembles black. Honey's shade is determined by a number of elements, including its botanical source, the ecological and other situations in which it is gathered, and the honey's age. Honey's colour has a significant role in determining its market value. Honeys with a lighter colour are typically used for direct consumption in the home, while honeys with a darker colour are typically employed for industrial purposes.

The Pfund scale, which is represented in the table below, is used to measure the colour of honey in millimetres. A chart was used in order to visually distinguish the colours of the honey samples. (Krell, 1996). The colors were recognized and the data of Pfund scale was noted in the record.

Pfund scale and color standard (USDA)

S. N.	Pfund SCALE (IN MM)	COLOR STANDARDS (USDA)
1	0 – 8	Colourless (as like water)
2	9 –17	Extra white
3	18 – 34	White
4	35 – 50	Light amber (Extra)
5	51 – 85	Light amber
6	86 – 114	Amber
7	≥ 115	Dark amber

Protein content estimation:

Using Ranganna's 2007 micro-Kjeldahl approach, researchers were able to estimate the food sample's nitrogen content. To go from nitrogen content to protein content, a suitable factor was applied to the nitrogen value. The Indian Standard Institute adopted the approach as a standard method for protein measurement. The procedure works by oxidizing the sample bisulphuric acid and then turning the nitrogen-containing chemicals into ammonium sulphate, which is another nitrogen-containing material. When alkali solution is supplemented to the ammonium sulphate solution, ammonia is liberated and collected by distillation into a known amount of suitable acid. By titrating the content with a standard alkali solution, the amount of acid that remained unused was determined.

Results and Discussions

Moisture content of honey

The honey sample had variability in moisture content in botanical and locational sources (table 1). Honey produced from eucalyptus had a water content that ranged from 16.33 percent to 17.17 percent, with a mean value of 17.17 percent (table 1). Honey samples from the Sohawal tehsil had a numerically greater value for their moisture percentage, whilst those from the Sadar tehsil had the lowest value. Honey moisture accounts for between 17.37 and 18.63 percent of the total honey samples obtained from Brassica throughout all of the tehsils (table 1). Honey samples collected from a variety of locations had considerably different levels of moisture content. Moisture percentage of honey samples from Bikapur and Rudauli was significantly higher than the Sadar tehsil. The moisture percentage of Honey samples obtained from Litchi were ranging from 19.07% and 20.17% (table1). When comparing honey samples from various regions of the country, there was no significant change in the moisture content. Honey samples from Bikapur tehsil exhibited a greater percentage of moisture than those from Sadar tehsil.

In the composition of honey, it is the second most bulk-forming agent both by weight and volume (Krell, 1996). Water is the solvent in which all other constituents of honey were got dissolved or maybe as the suspended particles. The aqueous content of honey may vary tremendously in the kind of honeys depending upon the numerous factors. It was revealed from the data that majority of samples have the moisture contents below 20%. According to the

standards set by BIS (Bureau of Indian Standards), the special grade honey should have the water content below 20 % (IS 4941, 2002).

Table 1: Regional variations in moisture content and pH in various floral origin of honey sample.

Sample location	Moisture content (%) Mean±SE			pH		
	Eucalyptus sp	Brassica sp.	Litchi sp.	Eucalyptus sp	Brassica sp.	Litchi sp.
Bikapur	16.75±0.22	18.63^{a}±0.27	20.17±0.27	4.0±0.12	4.07±0.30	3.90^{b}±0.31
Rudauli	17.05±0.25	18.45^{ab}±0.34	19.92±0.55	4.40±0.21	4.10±0.26	4.10^{ab}±0.10
Sohawal	17.17±0.59	18.22^{abc}±0.36	20.02±0.49	4.68±0.17	4.33±0.09	4.73^{a}±0.18
Sadar	16.33±0.30	17.37^{cd}±0.54	19.07±0.23	4.53±0.38	4.07±0.37	3.97^{bc}±0.12

Determination of pH of Honey

The pH of various honey samples collected from Eucalyptus were ranging from 4.00 to 4.68 (table 1). There were significant similarities found in the values of the pH of Eucalyptus honey samples collected from the Bikapur, Rudauli, Sohawal and Sadar tehsil of Ayodhya district. Honey samples collected from Eucalyptus in Sohawal tehsil recorded highest Ph value while that of Bikapur was found least. The pH of honey samples collected from Brassica at various geographical locations varies between 4.07 to 4.33 (Table 4.24). Honey samples collected in Sohawal tehsil recorded highest Ph value while that of Bikapur and Sadar was found to be lowest. The pH of honey samples collected from Brassica at various geographical locations varies between 3.90 to 4.73 (4.25). Honey samples collected in Sohawal tehsil had significantly higher Ph value compared to honey samples from Bikapur and Sadar tehsils.

The pH of honey is an indication of its acidic or basic nature. It was revealed in plenty of studies that honey is naturally acidic in nature. The locational variations or the differences in the botanical sources cannot change the nature of honey from acidic to basic (Atul Kumar *et al.,* 2018; Manu Kumar *et al.,* 2013).

1. **Free Acidity:**

The mean free acidity values in the honey samples collected from different tehsil of Ayodhya district in Eucalyptus, Brassica and Litchi origins are summerised in the table 2. It was varied from 12.50 to 32.50 meq/kg. The free acidity value in honey samples belonging to Eucalyptus, Brassica and Litchi flora of Bikapur tehsil were 24.50±1.61, 23.50±3.50 and 25.50±3.61 meq/kg respectively. The corresponding values of free acidity in Rudauli tehsil were 18.67±1.86, 23.67±3.32 and 22.17±1.14 meq/kg; 16.67±1.67, 20.00±1.16 and 16.83±1.17 meq/kg in sohawal tehsil; 19.67±4.05, 23.50±4.28±0.09 and 23.83±1.43 meq/kg in Sadar tehsil.The mean value of free acidity was reported higher in Brassica originated honey samples as compared to Eucalyptus and Litchi honey samples.

The average free acidity in all of the honey samples was below the upper limit of 50 meq/kg. The low acidity in honey samples indicated that there had been no unwanted

fermentation. Organic acids and inorganic ions contribute to honey's acidity. A similarity can be seen between the results of this research and the studies conducted previously by Gulfraz *et al.* (2010), Kayoed and Oyeyemi (2014), Khan *et al.* (2016), as well as Krishnasree and Ukkuru (2017). Only the Sohawal Brassica honeys (P0.05) varied from the Eucalyptus and Litchi honeys of the same site (P>0.05) in terms of their botanical origins. Khan *eal*. (2016) and Kumar *et al.* (2018) found that floral origin had a significant influence on free acidity estimate, but Sohaimy *et al.* (2015) found no significant variations in the results.

Acidity is needful criterions for assessment of fermentation of honey, differentiation the authenticity of unifloral honey and demarcation of honeydew and nector. Contrary to our findings, higher value of free acidity but within the desirable range was reported by Qamer *et al.* (2008) in four apisdorsata honey procured from the four different forest of Nepal.

Table 2: Regional variation in free acidity (meqkg^{-1}) content in honey sample

Sample location	Free acidity (meq/kg) Mean±SE		
	Eucalyptus sp	**Brassica sp.**	**Litchi sp.**
Bikapur	24.50±1.61	23.50±3.50	25.50^{a}±3.61
Rudauli	18.67±1.86	23.67±3.32	22.17^{ab}±1.14
Sohawal	16.67±1.67	20.00±1.16	16.83^{bc}±1.17
Sadar	19.67±4.05	23.50±4.28	23.83±1.43

Electrical Conductivity of honey sample:

The electric conductivity of honey samples of different tehsils of Ayodhya district are presented in table 3. Electric conductivity of honey samples existed between the 0.12 to 0.85 µs/cm. The mean value of electric conductivity of Eucalyptus honey was highest in Sadar tehsil (0.29±0.05 µs/cm), followed by Sohawal tehsil (0.25±0.04 µs/cm), Rudauli tehsil (0.24±0.02 µs/cm) and least in Bikapur tehsil ((0.16±0.03 µs/cm). In Brassica honey, the mean electric conductivity was highest in Rudauli(0.63±0.06 µs/cm), followed by Sadar(0.60±0.05 µs/cm), Bikapur(0.49±0.02 µs/cm) and lowest in Sohawal tehsil (0.47±0.04 µs/cm). In Litchi honey, the averaged electric conductivity was greatest in Rudauli as well as Sadar with a value of 0.81 µs/cm, followed by Bikapur with a value of 0.77 µs/cm and least in Sohawal with a value of 0.61 µs/cm. The overall mean of pooled data of Eucalyptus, Brassica and litchi honey was 0.24±0.04, 0.55±0.03 and 0.75±0.03 µs/cm, respectively.

In present study, all the honey samples irrespective to floral origin showed electric conductivity within the range of EU standard except 3 litchi samples (1 from Rudauli; 2 from Sadar). Our results on different floral honey samples studied were consonance with findings observed by Qamer *et al*. (2008), Gulfaz *et al*. (2010), Buba *et al*. (2013), Boussaid *et al.* (2014), Abselami *et al*. (2018), Azonwade *et al.* (2018), Kumar *et al.* (2018), Valdes-Silverio *et al.* (2018), Flores *et a* . (2019), Hailu & Belay (2020) and Kharkamni (2021). Contrary to these finding on electric conductivity, Balchandra and Joshi, (2021) reported lower values in blended raw honey samples. Furthermore, the higher values of electric conductivity were reported as 7.6 to 12.4µS/m in Nigerian honey (Lullah-Deh *et al.,* 2018), 130.2 to 667.4µS/cm in Portuguese honey (Sereia *et al*., 2017). Electric conductivity is an important factor for

verifying the physicochemical properties of honey samples (Serrano *et al*., 2004). Using this procedure, one may also identify the genuineness of honey that comes from a single kind of flower. The electrical conductivity of honey is affected by the ionizable acids and compounds, minerals, and salts that are present in the sample's aqueous solution. It is used to honey samples in order to ascertain their level of purity and quality.

The Eucalyptus honeys had significantly lower electrical conductivity than Brassica honeys in all the studied tehsils of Ayodhya. However, the Litchi honey of Bikapur tehsil had significantly higher electrical conductivity as compare with Brassica honey of Bikapur. The rest Litchi honeys were significantly similar with Brassica honeys in Rudauli, sohawal and Sadar tehsils of Ayodhya. Similarly, Kumar *et al*. (2018) reported significantly differed, while Gulfraz *et al*. (2010) found significantly similar electric conductivity among the different floral honeys. The variation in electric conductivity is mainly due to variation in floral source of nectar that is carried by the honeybees to construct and store the honey. Soil health also indirectly affects the electric conductivity via pollen sources. Ageing of the honey leads to change in the chemical characteristics of honey that alter the electric conductivity.

Table 3: Electrical conductivity (μScm^{-1}) in different floral origin of honey samples

Tehsil	Eucalyptus sp.		Brassica sp.		Litchi sp.	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	0.16±0.03	0.12 – 0.21	0.49±0.02	0.45 – 0.52	0.77±0.02	0.75 - 0.80
Rudauli	0.24±0.02	0.20 – 0.28	0.63±0.06	0.53 – 0.72	0.81±0.02	0.78 – 0.85
Sohawal	0.25±0.04	0.18 – 0.32	0.47±0.04	0.40 – 0.55	0.61±0.04	0.55 – 0.69
Sadar	0.29±0.05	0.24 – 0.38	0.60±0.05	0.50 – 0.65	0.81±0.03	0.75 – 0.85
Overall	0.24±0.02	0.12 – 0.38	0.55±0.03	0.40 – 0.72	0.75±0.03	0.55 – 0.85

Hydroxy methyl furfural (HMF):

Hydroxy methyl furfural (HMF), is a cyclic aldehyde molecule formed by the non-enzymatic browning reaction, which results in the breakdown of sugars. The reaction is caused by minerals, acids, and, most importantly, carbohydrates.

The results were tabulated in the table 4. The hydroxyl methyl furfural content (HMF) of honey samples of different tehsils of Ayodhya district are shown in table 10 and also depicted in graph 10. HMF content of honey samples was differed from 4.0 to 10.0. The mean value of HMF of Eucalyptus honey was highest in Rudauli with 8.57±0.50, followed by Bikapur with 8.33±0.35, Sadar with 7.78±0.29 and least in Sohawal tehsil with 7.62±0.21. The mean HMF content of Brassica honey was greatest in Bikapur (6.27±0.15), followed by Rudauli (6.17±0.60), Sohawal (5.50±0.40) and lowest in Sadar tehsil (5.33±0.87). In Litchi honey, the averaged HMF was greatest in Bikapur with 10.10±0.21, followed by Sohawal with 8.57±0.57, Sadar with 8.50±0.29 and least in Rudauli tehsil with 8.23±0.51. The overall mean of pooled data of Eucalyptus, Brassica and litchi honey was 8.08±0.19, 5.82±0.26 and 8.85±0.28, respectively.

The current findings of HMF content in Eucalyptus, Brassica and Litchi honey were close agreement with the observations reported by Hailu and Belay (2020) in monofloral and polyfloral honeys. Unlikely, the higher HMF values were mentioned in various floral and geographical honey samples (Kharkamni, 2021). Moreover, the lower HMF content was stated

by Flores *et al*., 2019.

HMF contents of all the floral originated honeys were significantly differed in Bikapur tehsil of Ayodhya. The HMF content of Eucalyptus honey had significantly lower tham the Lichi honey in Sohawal tehsil. Similarly, Kumar *et al*. (2018) and Tigistu *et al*. (2021) also reported significantly differed HMF concentration among the different floral honeys. All the samples had HMF content, within the acceptable range (<80 mg/kg) according to Codex Alimentarius, 2001. The HMF content according to EU and Ethopian standard is not acceptable beyond the 40 mg/kg. All the samples assure the EU, Ethopian, Codex and AGMARK standards. Honey above the 100 mg/kg HMF is not acceptable, indicated for adulteration with invert sugars.

Hydroxy methyl furfural (HMF) is a chemical molecule. it forms naturally through a slow process throughout the storage of honey and more speedly when honey is warmed. HMF comes in existence with the breaking of the simple sugars (fructose and glucose) (Bradbear, N., 2009). HMF quantity is controlled by a number of parameters, and so it serves as a warning sign of overheating and bad storage conditions (Ahmed *et al*., 2014). HMF content rises with temperature and ageing, according to numerous researches (Batinić & Palinić, 2014; Vahčić & Matković, 2009).

Table 4: Hydroxy methyl furfural (mg Kg^{-1}) in different floral origin of honey samples

Tehsil	**Eucalyptus sp.**		**Brassica sp.**		**Litchi sp.**	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	8.33±0.35	7.8 – 9.0	6.27±0.15	6.0 – 6.5	10.10±0.21	6.3 – 10.0
Rudauli	8.57±0.50	7.8 – 9.5	6.17±0.60	5.0 – 7.1	8.23±0.51	7.5 – 9.2
Sohawal	7.62±0.21	7.3 – 8.0	5.50±0.40	4.7 – 6.0	8.57±0.57	8.0 – 9.7
Sadar	7.78±0.29	7.3 – 8.3	5.33±0.87	4.0 – 7.0	8.50±0.29	8.0 – 9.0
Overall	8.08±0.19	7.3 – 9.5	5.82±0.26	4.0 – 7.1	8.85±0.28	6.3 – 10.0

Pfund values and Color

The value of Pfund scale and color of honey samples are mentioned in Table 5. The mean Pfund value of Eucalyptus honey was highest in Bikapur (49.33), followed by Rudauli (48.33), Sadar (45.00) and Sohawal tehsil (42.33). The average Pfund values of Brassica honey were highest in Bikapur and Sohawal as 43.33, followed by Sadar tehsil as 43.00 and lowest in Rudauli tehsil as 41.33 mm. All the Brassica honey samples had within the range of 35-50 mm in Pfund scale and possess extra light amber color. The mean Pfund value of litchi honey was maximum in rudauli (72.33), followed by Bikapur (67.33), Sohawal (66.67) and least in Sadar tehsil (65.00). All the Litchi samples have within the 51-85 Pfund scale and have light amber color. The overall mean value of Pfund scale of Eucalyptus, Brassica and litchi honeys was 46.25±1.10, 42.83±0.99 and 67.83±1.84 mm, respectively.

Sohaimy *et al.* (2015) in mono floral Yameni honey, Hailu and Belay (2020) in Schefflera abyssinica honey reported 56.40 and 53.10 mm respectively, which were higher than Eucalyptus and Brassica honey but lower than Litchi honey of this study. The current findings of Eucalyptus, Brassica and Litchi honey were lower than those reported as 151.08 (Pontis *et al.,* 2014) and 130.58 mm (Hailu and Belay, 2020) in poly floral honeys.

The Pfund scale value of Litchi honey had significantly greater ($P<0.05$) compare to both

Eucalyptus and Brassica honeys in all the studied locations of Ayodhya district of Uttar Pradesh. The Eucalyptus honeys had numerically higher Pfund but significantaly similar coloration in all the studied tehsils of Ayodhya. Similarly, Boussaid *et al.* (2014) reported significantly variation in Pfund values in different floral sourced honeys, while Sohaimy *et al.* (2015) observed significantly no differences in Pfund scale of various floral honeys.

Color of honey depends upon the chemical composition of honey, ageing of honey and storage temperature of honey. Mineral content present in the honey is the major factor to determine the honey color. Honey with high mineral content usually has dark color. Mineral content is proportionate to the ash content of honey. So the dark colored honey have greater ash content, while light colored honey have less quantity of ash. The fresh honey samples are lighter as compared to aged honey. Honey exposes to warm temperature results to dark in color with greater Pfund scale value.

Table 5: Pfund scale (mm) and color of different floral origin of honey samples

Tehsil	Eucalyptus sp.		Brassica sp.		Litchi sp.	
	Mean	Color	Mean	Color	Mean	Color
Bikapur	49.33±1.77	Extra light Amber	43.33±2.41	Extra light Amber	67.33±1.77	Light Amber
Rudauli	48.33±2.03	Extra light Amber	41.33±2.73	Extra light Amber	72.33±5.37	Light Amber
Sohawal	42.33±1.20	Extra light Amber	43.33±2.41	Extra light Amber	66.67±2.19	Light Amber
Sadar	45.00±1.53	Extra light Amber	43.33±1.77	Extra light Amber	65.00±4.73	Light Amber
Overall	46.25±1.10	Extra light Amber	42.83±0.99	Extra light Amber	67.83±1.84	Light Amber

Sugar content

2. The mean values of total sugar content of honey samples of different tehsils of Ayodhya district are shows in the table 6. It was ranged between 71.97 to 81.7 %. Total sugar content in Bikapur, Rudauli, Sohawal and Sadar tehsil was observed as 79.55±0.80, 78.45±0.73, 74.96±2.05 and 78.87±1.52%, respectively in Eucalyptus originated honey samples; 76.3±0.91, 74.00±1.16, 73.57±1.34 and 70.90±1.63 in Brassica originated honey samples; 73.57±0.83, 70.88±0.77, 71.18±1.18 and 72.23±2.52 in Litchi originated honey samples. The overall mean value of total sugar content was higher in Eucalyptus honey, intermediate in Brassica honey and lowest in litchi honey.

The present observations of total sugar content of honey samples were agreement with the findings reported by kumar *et al*. (2013) in four different kinds of Indian honey samples (64.88-73.08%), Fahim *et al*. (2014) in Ciddar honey samples (61.67- 72.42 %), Krishnasree and Ukkuru, 2017 in different honeybee's honey (71.6 -72.14%). Unlikely, the lower total sugar content were quoted by Khalil *et al*. (2012) in honey samples of western Maharashtra (67.03 ± 0.68 %) and Naga *et al*. (2020) in three regional honey samples (61.40% to 68.11%). Total sugar content of all the different floral honeys of Rudauli tehsil were differed

significantly (P<0.5). The difference of total sugar contents between Eucalyptus and Brassica honeys in Sadar tehsil was significantly more, while rest of honeys had significantly similar total sugar content. Khan *et al*. (2016) reported significant effect of floral sources on sugar content of honey. The variation in total sugar content of different honey samples might be due to variation in floral sources of nectar, soil and water content of sample collecting site, weather and climate variation, beekeeping practices etc.

Table 6: Total sugar (%) in different floral origin of honey samples

Tehsil	**Eucalyptus sp**		**Brassica sp.**		**Litchi sp.**	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	79.55±0.80	78-80.65	76.3±0.91	74.9-78	73.57±0.83	72.5-75.2
Rudauli	78.45±0.73	77-79.35	74.00±1.16	72-76	70.88±0.77	69.5-72.15
Sohawal	74.96±2.05	72-78.89	73.57±1.34	71.4-76	71.18±1.18	69-73.05
Sadar	78.87±1.52	76.5-81.7	70.90±1.63	68.5-74	72.23±2.52	69-77.2
Overall	77.96±0.80	72-81.7	73.52±0.76	68.5-78	71.97±0.72	69-77.2

Reducing Sugar Content

The reducing sugar in different samples of honey in present study ranged from 59.5 to 76.84 %; displayed in table 7. The highest value was acquired by theEucalyptus honey sample of Sadar tehsil, while lowest value was persist in the Brassica honey of Sadar tehsil as well as litchi honey of Rudauli tehsil. The mean reducing sugar value of Eucalyptus honey was greatest in the Sadar tehsil (72.25±2.47) followed by Bikapur (70.83±1.57), Rudauli (70.70±1.39) and lowest in Sohawal tehsil (68.75±3.00). The average reducing sugar content of Brassica honey was highest in Bikapur tehsil (68.34±1.54), followed by Sohawal (64.40±1.07), then Rudauli (63.07±0.81) and least in Sadar tehsil (61.87±1.23). Mean reducing sugar of Litchi honey was maximum in Sadar tehsil (65.13±2.39), followed by Bikapur tehsil (64.65±1.19), then Sohawal (63.00±0.76), and minimum in Rudauli tehsil (61.43±1.46).

Majority of the honey samples had more than 60% total reducing sugars, reflected a good characteristics of honey. Likewise to our findings, Khalil *et al*. (2012) in Algerian honey samples, Kumar *et al.* (2013) in Indian honey, Bhattarai *et al*. (2019) in different floral origin honey samples. Conversely to current observations, Kinoo *et al.* (2012), Nega *et al.* (2020) stated lower reducing sugar contents.

All the floral honey samples have significantly similar reducing sugar. The Eucalyptus honeys had significantly higher reducing sugar as compare with Brassica honeys in Rudauli and Sadar tehsil of Ayodhya. Boussaid *et al*. (2014) and Tigistu *et al*. (2021) reported significantly differed reducing sugar content in different botanically origin honeys.

Table 7: Reducing sugar (%) in different floral origin of honey samples

Tehsil	**Eucalyptus sp.**		**Brassica sp.**		**Litchi sp.**	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	70.83±1.57	68.3 – 73.7	68.37±1.54	65.3 – 70.0	64.65±1.19	63.0 – 66.95
Rudauli	70.7±1.39	68.0 – 72.6	63.07±0.81	61.7 – 64.5	61.43±1.46	59.5 – 64.3
Sohawal	68.75±3.00	65.5 – 74.74	64.4±1.07	62.5 – 66.2	63±0.76	61.5 – 64.0
Sadar	72.25±2.47	68.4 – 76.84	61.87±1.23	59.5 – 63.6	65.13±2.39	62.5 – 69.9

Overall	70.63±1.01	65.5 – 76.84	64.26±0.87	59.5 – 70.0	63.55±0.80	59.5 – 69.9

Non-Reducing Sugar (Sucrose)

The non-reducing sugar content of different honey samples is differed from 4.15 to 14.30 % (table 8). The greatest value of non-reducing content was received by the Brassica honey of Rudauli tehsil, while smallest value was confirmed in the Eucalyptus honey of Sohawal tehsil. The mean reducing sugar value of Eucalyptus honey was differed from 6.22±1.12 (Sohawal tehsil) to 8.72±0.89 (Bikapur tehsil). Mean non-reducing sugar of Litchi honey was ranged from 7.10±0.31 (Sadar tehsil) to 9.45±0.81 (Rudauli tehsil). The overall means of Eucalyptus, Brassica and Litchi origin honey were 7.33±0.49, 9.46±0.56 and 8.41±0.36, respectively.

The current findings of non-reducing sugar were more or less similar to earlier observations (Iglesias *et al* . (2012); Nega *et al*. (2020). Unlikely, the higher non-reducing sugar was reported by Kaushik (1988). Furthermore, Fahim, (2014) quoted lower value of non-reducing sugar in Ciddar honey.

Non-reducing sugar content of all the honey samples of different floral origin was observed significantly similar. Unlikely, Tigistu *et al*. (2021) found significant differed non-reducing content in different botanical sourced honeys in different parts of world.

Non-reducing sugar concentrations may vary because of the variety of floral sources used by honeybees to collect pollen nectar; differentiation in geographical co-ordinates i.e. latitude and longitude of sites; differences in beekeeping practices; storage duration of collecting samples leads to change in physicochemical properties; different varieties of honeybees altering the physicochemical characteristics of honey sample by their specific honey processing.

Table 8: Non-Reducing sugar (%) in different floral origin of honey samples

Thesil	**Eucalyptus sp**		**Brassica sp.**		**Litchi sp.**	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	8.72±0.89	6.95-9.7	7.93±1.05	6-9.6	8.92±0.55	8.25-10
Rudauli	7.75±0.66	6.75-9	10.93±1.97	7.5-14.3	9.45±0.81	7.85-10.5
Sohawal	6.22±1.12	4.15-6.5	9.17±0.32	8.8-9.8	8.18±0.46	7.5-9.05
Sadar	6.62±0.95	4.86-8.1	9.03±0.78	7.7-10.4	7.10±0.31	6.5-7.5
Overall	7.33±0.49	4.15-9.7	9.46±0.56	6-14.3	8.41±0.36	6.5-10.5

Total protein content

The protein content for Eucalyptus, Brassica and Litchi honey samples in different tehsils of Ayodhya district are summarized in table 9. Protein content in different samples varied from 0.25 – 1.01% i.e. 0.25-1.01 g/100g honey. The mean value of protein content in Eucalyptus honey was highest in Bikapur (0.98±0.02), followed by Rudauli (0.88±0.02), Sadar (0.85±0.0.04) and least in Sohawal tehsil (0.78±0.06 g/100g). In Brassica honey, the highest protein was in Sohawal (0.77±0.07), followed by Bikapur (0.68±0.07), Rudauli (0.55±0.02) and lowest in Sadar tehsil (0.38±0.04 g/100g). In Litchi honey, maximum protein content in Sadar (1.00±0.23), followed by Rudauli (0.45±0.03), Sohawal (0.43±0.04) and minimum in Bikapur (0.41±0.02 g/100g). The overall mean of pooled data of Eucalyptus honey was 0.87±0.03. However in Brassica honey was 0.74±0.19 and in

litchi honey was 0.72±0.31 g/100g.

Table 9: Protein content (g/100g) in different floral origin of honey samples

Thesil	Eucalyptus sp		Brassica sp.		Litchi sp.	
	Mean	Range	Mean	Range	Mean	Range
Bikapur	0.98±0.02	0.94-1.01	0.68±0.07	0.60-0.81	0.41±0.02	0.38-0.45
Rudauli	0.88±0.02	0.86-0.92	0.55±0.02	0.50-0.58	0.45±0.03	0.40-0.50
Sohawal	0.78±0.06	0.68-0.87	0.77±0.77	0.51-0.28	0.43±0.04	0.37-0.50
Sadar	0.85±0.04	0.80-0.94	0.38±0.04	0.33-0.46	1.00±0.23	0.25-0.41
Overall	0.87±0.03	0.80-1.01	0.74±0.19	0.33-0.81	0.72±0.31	0.25-0.50

As compared to current findings, the lower protein contents were reported by Escuredo *et al.* (2013) in Spanish unifloral honey, Islam *et al.* (2014) in litchi honey 0.52 – 0.53 g/100g), Sohaimy *et al.* (2015) in honey from various origins (0.169 – 0.467 g/100g), Nguyen *et al.* (2018) in Manuka honey of New Zealand (0.13 g/100g) and Hailu and Belay, (2020) in Schefflera abyssinica (0.43 g/100g) and polyfloral honey (0.51 g/100g). However, Sajwani *et al.* (2007) mentioned higher protein content (0.75 – 5.18 g/100g) in 51 honey samples belonging to unifloral and multifloral origin. The protein content of all the floral sourced honeys were significantly ($P<0.05$) differed in Bikapur and Rudauli while non-significant in Sohawal and Sadar tehsil of Ayodhya. Likely, Gulfraz *et al*. (2010) and Kumar *et al*. (2018) observed significant differences, while Boussaid *et al*. (2014) and Sohaimy *et al*. (2015) quoted non-significant differences in protein contents in different floral honeys. It is possible that a similar forage plant flora exists if the majority of honey samples have the same amount of protein, regardless of the sort of honey, the season, or the bees that produced the honey (Helrich, 1990).

Conclusion

This investigation was to study the physiochemical characteristics of different samples of honey of different origins collected from four tehsil of Ayodhya district, Uttar Pradesh, India. The moisture content and pH of collected sample within the standard limit of moisture content (< 25%) and pH value. Free acidity, electrical conductivity, Hydroxy methyl furfural content, Pfund scal, total sugar, reducing and non-reducing sugar and protein content were observed from Bikapur, Rudauli, Shawal and Sadar Tehsil of Ayodhya district from Eucalyptus, Brassica and Litchi flower sources. The present study concludes that physicochemical properties of honey were varied based on the basis of geographical location and botanical origins.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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