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Evaluation of the physicochemical qualities and antioxidant properties of some Bangladeshi varieties of honey: A comparative study

Sabrina Trisha [a], Md. Golam Mortuza [a], Juwel Rana [b,*], Kazi Hizbul Islam [a], Zannatul Ferdoush [b], Raiya Adiba Antora [c], Sarif Istiak Akash [c], Mohammad Gulzarul Aziz [c], M. Burhan Uddin [c]

[a] Department of Biochemistry and Molecular Biology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[b] Department of Nutrition & Food Engineering, Daffodil International University, Daffodil Smart City, Birulia, Savar, Dhaka 1216, Bangladesh
[c] Department of Food Technology and Rural Industries, Faculty of Agricultural Engineering and Technology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

1. Introduction

Honey is a viscous liquid that is a complex creation that is made up of the nectar of many flowers that is gathered from the honeycomb that is formed by honey bees (*Apis mellifera*) [1,2]. Honey has been used for thousands of years, and because to its many health advantages and the discovery of over 200 bioactive compounds, its popularity has only increased in recent years [3].

The antioxidant properties of honey may be affected by polyphenols, acids, catalase peroxidase, and browning reactions [4]. A large number of plant polyphenols are simple phenolic compounds, which are cited as the primary antioxidants in various types of honey. Honey's strong antioxidant content is due in large part to the high quantities of phenolic derivates found in it, including phenolic acids and flavonoids [5]. Honey has attracted many researchers as potential functional food, herbal, and aromatherapy product [6]. Researchers have reported that honey is recommended to children, sportspersons, and older people for overall well-being due to its potential medicinal properties [7].

It plays a pivotal role in the production of a huge range of items, including alcoholic beverages, energy drinks, baked goods, sweets, and bars, among many others. It is one of the most extensively used non-food ingredients in medicines for wound healing, antibacterial, anti-inflammatory, anti-diabetic, antioxidant, anti-tumoral, anti-allergic, anti-browning, anti-parasite, and anti-viral [8]. Honey is frequently contaminated and sold under a false name or adulterated to make money [9]. Parameters, including water content, mineral content, the ratio of reducing to non-reducing sugars, free acidity, and the capacity to conduct electricity (EC), are used by the European Council to classify the quality of honey [10]. When the criteria mentioned above are considered, it is possible to assess the quality of the honey.

Bangladeshi culture has traditionally used it as food and medicinal purpose. The honey production in the fiscal year of 2019–2019 was 0.3 million kg, whereas the 0.45 million kg during the fiscal year of 2020–2021. A compound annual growth rate of 6.2 % is predicted for the honey market in Bangladesh between 2020 and 2026 [11]. This growth is attributed to an increase in rising health consciousness among consumers and a growing preference for fresh food products.

The agrarian nation of Bangladesh struggles with poverty and unemployment. Beekeeping is a vital source of employment and revenue for rural areas. This significant sector is plagued by adulteration and mono and poly-flora honey mislabelling. Despite its relevance, Bangladesh has limited research articles on honey bee pollen chemical composition and bioactivities.

The current study's objectives were to examine the floral origin, moisture content, pH, total soluble solids (TSS), electric conductivity (EC), total sugars, total acidity, hydroxymethylfurfural (HMF), phenolic compounds, and the radical scavenging activity of honey samples from Bangladesh's most popular honey varieties (Rubber, Mustard, Black cumin, Litchi, and Sundarbans). In addition, a correlation between all of the examined factors was explored.

2. Materials and methods

2.1. Sample and chemicals

Five varieties of raw honey of Bangladesh Rubber (*Hevea brasiliensis*), Mustard (*Brassica juncea*), Black cumin (*Nigella sativa*), Litchi (*Litchi chinensis*), Sundarbans (*Aegiceras corniculatum*) were collected, and brought to the laboratory. Before they were analyzed, kept in sealed glass containers at ambient temperature until they were needed (Fig. 1). Within two months of receiving the samples, all the tests were completed. All of the reagents and chemical substances that were used were of a grade suitable for analytical usage.

* Corresponding author.
E-mail address: juwel.nfe@diu.edu.bd (J. Rana).

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2.2. Identification of melissoplaynological characteristics of honey samples

Honey samples were characterized using melissopalynological analysis, and then classed according to the specific botanical variety that each sample represented Honey is said to be monofloral when more than 45 % of its pollen comes from a single kind of flower, as stated by Ref. [12].

2.3. Evaluation of the physicochemical qualities

2.3.1. Moisture content

The refractometric technique ((Erma Refractometer, India)) was used to calculate the amount of moisture present. After determining the refractive indices of the samples while they were at room temperature, the researchers used the 0.00023/°C correction factor to adjust the results such that they were accurate for the reference temperature of 20 °C. The percentage of the material's moisture content was determined by utilizing Wedmore's table [13].

2.3.2. pH determination

The pH was determined with the use of a pH meter (HI122, Hanna instruments, USA) that had been calibrated before the experiment. According to Ref. [14] research, a solution of 10 g of honey was homogenized after being diluted in 75 mL of distilled water.

2.3.3. Total soluble solids (°Brix)

The total soluble solids (°Brix) were measured by refractometric analysis (Erma Refractometer, India) from 0 to 90° [15].

2.3.4. Color determination

With the use of a Minolta Chroma Meter (Model CR-400, Minolta, Japan), the honey's exact hue was identified. The hue of honey was studied and documented by the International Commission on Illumination CIE. The colors of honey samples were represented using the L*, a*, and b* color space coordinate system, where L* (Lightness) equals 100 for white and 0 for black; a* (redness/greenness axis) equals positive values; and b* (yellowness/blueness axis) equals negative values [16].

2.3.5. Electrical Conductivity (EC) and Total Dissolved solids (TDS) Measurement

EC and TDS were measured using a conductivity meter (HI 98311, Hanna Instruments, Mauritius) for honey solution (20 % w/v) made by dissolving honey in distilled water and pouring it into a 100-mL volumetric flask. To calculate the EC and TDS of each sample, we submerged the conductivity probe in the specimen's solutions; the values were then converted to mS/cm and ppm [17].

2.3.6. Hydroxymethylfurfural (HMF) concentrations

Honey samples were tested for HMF content using the International Honey Commission's White technique. A UV–Vis spectrophotometer assessed the absorbing capacity at 284 nm spectrum of a cleaned aqueous honey solution against a control solution [18]. The following calculation calculated honey's HMF content:

$$\text{HMF (mg / kg of honey)} = \frac{(A_{284} - A_{336})\text{xFactor}}{W}$$

Where,

W = Honey sample weight (g).

Factor = $\frac{126 \text{ x } 1000 \text{ x } 1000}{16830 \text{ x } 10} = 748.66$

126 = Molecular weight of HMF.

1000 = Scale from grams (g) to milligrams (mg).

10 = Converting 5–50 ml.

1000 = Honey weight conversion to kg.

16,830 = The absorption coefficient of HMF at 284 nm in molar units.

2.3.7. Diastase activity determination

Diastase activity was determined by an established spectrophotometric method by International Honey Commission. Using the recommended AOAC procedure, the DN or diastase number was calculated as 300 divided by the time required to achieve an absorbance value of 0.235 [15]. The diastase activity was quantified using the resulting diastase number (DN):

$$\text{DN} = \frac{60\text{minutes}}{t_x} \times \frac{0.10}{0.01} \times \frac{1.0}{2.0} = \frac{300}{t_x}$$

Where, t_x = reaction time in minutes.

2.4. Evaluation of antioxidant levels

2.4.1. Proline concentration

After the ninhydrin was applied, a color comparison was performed with a standard for proline to determine the amount of proline present. The information was reported as a percentage of the total mass of the honey expressed in milligrams per kilogram [14]. Proline concentration was calculated as follows:

$$\text{Proline(mg / kg)} = \frac{E_s}{E_a} \times \frac{E_1}{E_2} \times 80$$

Where,

E_s = A specimen solution absorption

E_a = Absorption of the proline reference solution (mean of two measurements).

E_1 = The amount of proline used in the reference solution (mg).

E_2 = Grams of honey

80 = A factor of dilution.

Fig. 1. Five honey specimens in sealed glass containers at room-temperature.

2.4.2. Total phenolic content (TPC) determination

Honey samples were tested for the presence of phenolic compounds using a modified version of the spectrophotometric Folin-Ciocalteu procedure [19]. The following equation obtained the results:

$$\text{TPC(mg GAEs / kg)} = \frac{C_{GA} \times V \times DF \times 100}{\text{weight of honey sample (g)}}$$

Where,

C_{GA} = Standard curve gallic acid concentration (mg/ml).

V = The volume of the honey solution (ml).

DF = A factor of dilution.

2.4.3. Total Flavonoids determination

A colorimetric method using standard solutions of catechin was used in order to provide an accurate reading of the total flavonoid concentrations in the samples. According to Zhishen et al. [20] the findings were reported in terms of milligrams of catechin equivalents (CEQ) per kilogram of honey.

2.4.4. Antioxidant activity (DPPH- free radical-scavenging activity determination

DPPH radical scavenging activity was also used in the research [21] in order to investigate the antioxidant properties of the honey samples. The amount of the DPPH radical that was reduced was measured by looking at the absorbance of the sample at 517 nm [22]. As a standard material, butylated hydroxytoluene, also known as BHT, was used. The equation that was used in the calculation of the radical scavenging activity (RSA) is as follows:

$$\%\ \text{RSA} = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100$$

Where,

A_{DPPH} = Stands for the DPPH solution's absorbance

A_S = absorbance of the solution after a particular amount of sample solution has been added.

The IC_{50} value was determined to be the sample concentration that was necessary to block 50 % of DPPH free radicals by its calculation. This was accomplished by visually graphing the percentages of DPPH scavenging activity versus the specimen concentrations.

2.5. Statistical and multivariate data analysis

All the samples were measured in triplicate to obtain accuracy. A one-way univariate analysis of variance (ANOVA) was carried out in order to make a direct comparison between the various honey samples' mean values for the physicochemical and antioxidant characteristics. Tukey's test was used to determine whether or not there were statistically significant differences between the means at a level of confidence of 95 % to calculate the association between various characteristics, correlation analysis was performed. The results were expressed via the use of Pearson correlation coefficients (r), which were significant at $p < 0.05$ and $p < 0.01$, respectively. IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) software was used to perform these analyses.

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out with the use of statistical software developed by Minitab (Version 18.0, Minitab Inc., USA) in order to categorize the samples according to the physicochemical and antioxidant characteristics that they had. HCA and PCA analyses were performed on the chosen variables. The honey specimens were categorized by means of HCA according to the physical-chemical and antioxidant properties that they had. The sample similarities were calculated using Euclidean distance with a complete linkage rule, and clusters were formed using a hierarchical agglomerative process. PCA was used to classify the honey based on its antioxidant potentiality.

3. Results and discussion

3.1. Physicochemical characteristics

Melissopalynological analysis of the specimens revealed that their pollen content ranged from 23 % to 89 %, depending on the kind of plant they were. Specifically, *Hevea brasiliensis* was the predominant pollen in rubber honey (49–77 %), *Brassica juncea* in mustard honey (74–89 %), *Nigella sativa* in black cumin honey (56–72 %), *Litchi chinensis* in litchi honey (47–56 %), and *Aegiceras corniculatum* in Sundarbans honey (23–38 %). Except for Sundarbans honey, all samples were characterized by their monofloral nature.

The temperature and relative humidity of the climate in a particular region may have a significant impact on the level of moisture of honey. In certain cases, the beekeeping conditions in honey colonies can also have an effect on the moisture content of honey. Sometimes the harvest period also affects honey's moisture [23]. It is of the utmost importance to ascertain the amount of moisture that honey contains as it indicates the honey's quality and offers stability and resistance against microbial growth. Within the scope of this investigation, the moisture levels of the obtained specimens of honey were analyzed and given in Table 1. The lowest moisture content (16.64 %) was exhibited by Litchi Honey (LH) and the highest (19.84 %) by Mustard Honey (MH); both were lower than the maximum limit based on the requirements of the worldwide standard for honey [24]. As the moisture percentage in honey indicates storage stability, it could be assumed that all the honey samples possessed good storage stability. In addition to moisture, pH is another factor that affects the consistency of honey when it is being stored as well as the development of microorganisms [25]. According to this study's pH tests, the honey samples were acidic, with a pH value between 3.83 and 4.64. These findings were similar to the previous research on Bangladeshi honey [26].

The total soluble solids of the honey specimens were evaluated using a refractometer, and the findings were represented in degrees Brix and varied from 76.08 ± 0.1 for RH to 83.41 ± 0.5 for SbH (Table 1). The obtained total soluble solids indicate the amount of dissolved solids in honey. The total solid content also helps determine any honey adulteration [27]. The Sanford Honey Judging and criterion was created since the Codex Alimentarius Commission does not have a maximum or minimum total soluble solids criterion for honey. Honey Judging and Standards classifies high-grade honey as having a total soluble solid of 81.4 % or more, whereas lower-grade honey has 80–81.3 % [28]. Based on the studies and standards, BcH and SbH samples were of higher grade (82.02 ± 0.06 and 83.41 ± 0.52, respectively).

The color of honey is the first aspect of its quality, as it influences the

Table 1
Physicochemical parameters of five Bangladeshi honey samples.

Sample code	Moisture (%)	TSS (°Brix)	PH	Electrical conductivity (mS/cm)	TDS (ppm)	HMF (mg/kg)
LH	16.64 ± 0.05^{a}	78.04 ± 0.04^{b}	4.2 ± 0.06^{b}	0.65 ± 0.001^{c}	219 ±1^{d}	22.28 ± 0.31^{b}
MH	19.84 ± 0.16^{c}	79.47 ± 0.50^{c}	4.64 ± 0.06^{c}	0.36 ± 0.001^{a}	54±1^{a}	78.80 ± 0.26^{e}
RH	18.47 ± 0.46^{b}	76.08 ± 0.10^{a}	4.14 ± 0.08^{b}	0.38 ± 0.001^{b}	57±1^{b}	46.28 ± 0.62^{d}
BcH	18.29 ± 0.47^{b}	82.02 ± 0.06^{d}	4.53 ± 0.02^{c}	0.37 ± 0.001^{a}	189 ±1^{c}	24.66 ± 0.44^{c}
SbH	17.08 ± 0.17^{a}	83.41 ± 0.52^{e}	3.83 ± 0.06^{a}	0.79 ± 0.011^{d}	260 ±1^{e}	14.81 ± 0.28^{a}

Means were compared by using a one-way ANOVA with post hoc multiple Comparisons. Data were represented with mean ± SD ($p < 0.05$).

price and acceptance of honey in the market. Honey's hue reveals the existence of pigments in the substance. Many varieties of honey are available from various origins with color differences, from nearly transparent white to dark red and shades of yellow and amber. The formation of color mainly depends upon the botanical origin, temperature, storage time, and composition of nectar [29]. In this study, the color of honey was observed by the reflectance method with tristimulus values L*, a*, and b*. The L*, a*, and b* each had values that ranged from 37.6 to 46.23, 0.20–6.83, and 6.92–8.90, respectively (Table 2). The LH sample (L* = 46.23) had the significantly highest lightness, and RH (L* = 37.6) had the lowest lightness. The highest redness (a* = 6.83) and yellowness (b* = 8.90) were found in BcH and RH samples, respectively. The MH sample exhibited the least amount of redness (a* = 0.2) and yellowness (b* = 6.92), respectively, as shown in Fig. 2. Light honey, with a L* value more than 50, is distinguished from dark honey, with a L* value of 50 or less [30]. Using this definition, all of the honeys in this study fall under the category of "dark honey". The obtained values of color were found to be within the range of the previously studied variety of Iranian honey and 14 samples of Mexican honey [31,32].

The EC and TDS are normally measured together, where TDS stipulates the total amount of organic and inorganic substances in honey. It also indicates honey's ionized, micro-granular, molecular, or suspended state [26]. The lowest TDS value was 54 ppm for MH while the highest was 260 ppm for SbH (Table 1). The highest EC and TDS were found in SbH (0.79 mS/cm and 260 ppm) and the lowest in MH (0.36 mS/cm and 54 ppm). The EC changes depending on the botanical origin of the honey, and the resultant conductivity increases in direct proportion to the honey's TDS level [13]. The EC depended upon the organic acid, minerals, certain complex carbohydrates, and protein contents of different honey specimens [33,34]. According to the findings of this research, the sample consisting of SbH had the greatest EC (0.79 ± 0.01 mS/cm), whereas the sample consisting of MH had the lowest EC (0.36 ± 0.001 mS/cm) (Table 1). The honey samples' electrical conductivity (EC) values varied from 0.36 to 0.79 mS/cm, which is within the permissible limit of 0.8 mS/cm as stated in the European standard [24]. A research conducted by Bangladeshi researcher also found also samples varied between 0.2 and 0.8 mS/cm [26]. According to the findings of the above study, a positive connection between EC and TDS is an effective measure of the purity of honey. The SbH sample was found to have the highest quality according to the correlation, which can be supported by previous studies reported on other honey varieties from different countries [26, 35]

The HMF in honey is regarded to be a sign of how recently the honey was produced. Within the scope of this investigation, the HMF concentrations found in each of the samples varied from 14.80 mg/kg to 78.80 mg/kg (Table 1). The highest for MH, 78.80 ± 0.26 mg/kg, and the lowest for SbH, 14.8 ± 0.28 mg/kg. According to different studies, the HMF content is not a part of fresh honey and can be influenced by several parameters, for example, temperature, heating time, and storage conditions [36]. In addition to this, the amount of HMF might become elevated as a result of improper storage conditions and excessive heating [33]. In some previous studies, it was found that HMF content could be high in honey samples when the honey was being fabricated by adding inverted syrup. In the presence of acids, sugars may be heated to make HMF via a process known as inversion, which results in sucrose being converted into HMF [37]. So, it is important to have the HMF content within the allowed limit, which is lower than 80 mg/kg, indicating honey's originality and freshness [24]. Despite being kept at room temperature (20–25 °C), the HMF concentration in these samples was low. Bangladeshi honey may be ideal for long-term preservation because of its low pH and moisture content, which may reduce HMF generation.

Diastases are enzymes naturally found in different types of honey. These enzymes denature at high temperatures, and assessment of diastase content can give information about any overheating of honey or poor storage conditions because the enzyme activity would be reduced upon overheating or exposure to higher temperatures during storage [38]. As a result, along with the HMF, the freshness of honey can also be determined by the presence of the diastase enzyme. Extremely low or high diastase activity in honey may be a sign of spoilage or acid production, respectively [39]. Diastase activity is measured in Gothe, where one Gothe is equal to the quantity of enzyme needed to convert 0.01 gm of starch to the end point in 1 h at 40° Celsius. All of the honey samples included in this analysis had diastase levels between 32.6 and 167.24 Gothe/gm, much below the threshold set by legislation (minimum 8.0 Gothe units) (Table 1). Significantly the highest value was found for SbH (167.24 ± 1.66 Gothe/gm) and the lowest for MH (24.91 ± 1.62 Gothe/gm).

3.2. Antioxidant contents

In natural honey, many antioxidant compounds are found, including polyphenols, flavonoids, organic acids, amino acids, products of the Maillard reaction, different enzymes, carotenoids, etc. Honey contains several compounds, but polyphenols and flavonoids are the most important for their antioxidant capabilities and long-term health benefits [40,41]. The flavonoids found in honey are phenolic compounds with low molecular weight. They generally originated from nectar, pollens, or propolis, which is responsible for their aroma and antioxidant properties.

The highest phenolic content was recorded by sample SbH (489.8 ± 1.4 mg GAEs/kg), suggesting its higher antioxidant potential and the lowest was found at 242.12 ± 2.6 mg CEQ/kg for MH. The flavonoid content of SbH was the greatest, whereas that of MH was the lowest. The total phenolic content of the several samples of honey used in this research showed striking variations from one another which may be due to their distinct botanical and geographical origins. Researchers in Bangladesh examined eight different honey samples. They found that the phenolic contents were between 152.4 and 688.5 mg GAEs/kg, and flavonoid concentrations were 36 and 155 mg CEQ/kg, consistent with our findings [26]. Previous studies mentioned that honey's botanical and geographical regions affected the antioxidant activities, phenolic and flavonoid concentrations, and pollen distribution [42]. In addition, a number of earlier research indicated that the color of honey is linked to its phenolic content. Dark honey was shown to have greater levels of phenolics than light honey, while light honey had lower levels [43].

Based on this finding, we can stipulate that all samples in this study had a significant amount of total phenolic content. In every one of the honey samples, the levels of polyphenols were greater than the levels of

Table 2
Total levels of proline, total phenolic content, flavonoids, DPPH for the antioxidant capacity of five Bangladeshi honey samples.

Sample code	Diastase activity (Gothe/gm)	Proline (mg/kg)	TPC (mgGAEs/Kg)	Flavonoids (mgCEQ/kg)	Antioxidant activity	
					Inhibition (%)	$1/IC_{50}$ μg
LH	100.64 ± 1.53^{c}	867.13 ± 502.8^{ab}	399.1 ± 1.9^{d}	62.20 ± 0.83^{d}	45.732	70.88
MH	24.91 ± 1.62^{a}	768.59 ± 59.1^{ab}	242.12 ± 2.6^{a}	31.24 ± 0.71^{a}	30.182	232.14
RH	97.09 ± 1.60^{c}	413.86 ± 118.2^{a}	259.98 ± 1.3^{b}	39.93 ± 0.71^{b}	31.334	197.06
BcH	32.6 ± 1.62^{b}	748.88 ± 90.3^{b}	267.19 ± 2.4^{c}	57.93 ± 0.60^{c}	36.151	111.13
SbH	167.24 ± 1.66^{d}	1714.54 ± 591.2^{b}	489.80 ± 1.4^{e}	67.84 ± 0.19^{e}	56.23	46.65

Means were compared by using a one-way ANOVA with post hoc multiple Comparisons. Data were represented with mean ± SD ($p < 0.05$).

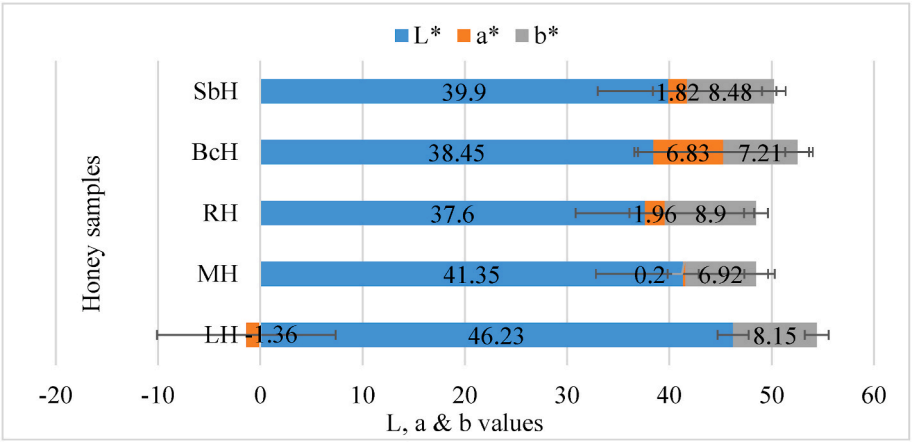

Fig. 2. The hues of five different types of honey from Bangladesh.

flavonoids (Table 2). The number of flavonoid concentrations in all the samples was similar to previous studies on different varieties [26,44].

All the honey samples were tested for their proline contents (Table 2). The measurement of honey's proline content is a very crucial criterion for determining the product's overall quality. Proline is a crucial amino acid that mostly originates from bees' saliva while transforming nectar into honey. The proline in honey may be used as a quality indicator, with higher concentrations indicating that the honey is at an optimal point of maturity and flavour. The proline content of SbH honey samples was the highest value (1714.54 ± 591.2 mg/kg) (Table 2). There is a possibility that the high proline concentration found in honey contributes to the antioxidant activity of honey [40].

The honey samples were assessed for their radical scavenging activities by DPPH assay. According to research, honey's antioxidant effects are mostly due to its flavonoid and phenolic content, hence DPPH is a stable nitrogen-centered radical employed to analyze them [42]. A higher DPPH scavenging activity indicates a higher quality of honey. The range of DPPH radical scavenging activity was from 30.18 % to 56.23 % (Table 2). DPPH radical scavenging activities were tested at concentrations of 20, 40, 60, 80, and 100 mg/ml; the maximum inhibition % was seen at the 100 mg/ml concentration for all of the samples (Fig. 3). SbH had the largest percentage of inhibition at 74.21 % percent, whereas MH had the lowest proportion at 35.20 % (Fig. 3). This study's results are consistent with prior research that indicated a range for Bangladeshi honey from 33.6 % to 97.5 % [26] (Islam et al., 2012).

In addition, the antioxidant activity was measured based on the scavenging activity against the free radical DPPH using the $1/IC_{50}$ criterion. This value represents the material's concentration that must be reached in order to block 50 % of free radicals. As a result, a lower $1/IC_{50}$ ratio in honey shows that it contains more antioxidants and has the

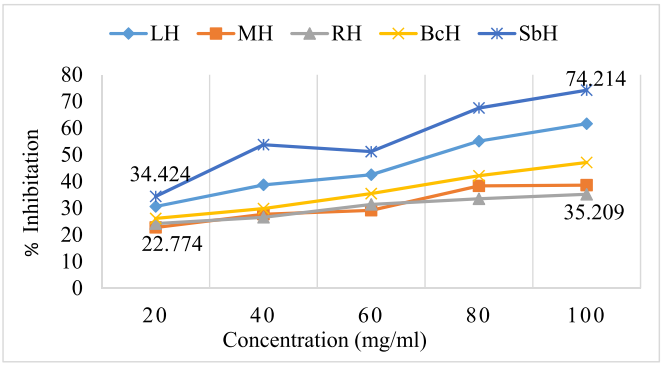

Fig. 3. Linear presentation of percentage inhibition of DPPH at different concentrations of all honey samples.

capacity to quench free radicals [45]. The SbH sample (46.65 μg) fulfilled all the desirable criteria.

3.3. Honey's physicochemical features and their connection to its antioxidant activity

Some remarkable relationships between physicochemical characteristics and antioxidant content were identified (Table 3). Significantly high levels of association ($r = 0.987$) between DPPH and TDS were found ($p < 0.01$ significance threshold). TDS value increases when DPPH is increased. When compared to other types of honey, Sundarbans honey (SbH) has the highest antioxidant content and the highest TDS value. This data indicates that the measured antioxidant levels of honey specimens may be affected by the honey's total dissolved solids. Researchers from Bangladesh tested various honey samples in 2012 and found a correlation between TDS and DPPH in those samples [26]. Again, a favorable correlation was observed between DPPH and HMF, pH, and moisture contents at 0.942, 0.625, and 0.916, respectively. According to the correlation, the honey samples which had more moisture, for example, the sample MH (19.84 ± 0.1) had less antioxidant potential activity means higher IC_{50} values (232.14 μg). A similar observation was found for Malaysian honeys [36].

The proline contents could also be observed as positively correlated with antioxidant potential (Table 3); the higher the proline content, the greater the antioxidant potential. Proline was found to have antioxidant capabilities, and several studies found a favorable correlation between proline and antioxidant activity in Indian honeys [35]. A notable negative linear correlation was noticed between pH and EC, pH and TPC, and HMF and TDS at −0.911, −0.878, and −0.895, respectively. The HMF and TDS showed a significant inverse correlation ($r = -0.895$), indicating that total dissolved solids increased with the decrease of HMF (Table 3). The EC, pH, and TPC showed a negatively significant correlation at (−0.911) and (−0.878), respectively. Bangladeshi researchers observed a negative correlation between pH and TPC in honey samples [26]. The total phenolic concentration of the honey specimens revealed that phenolics were the most important ingredient contributing to the radical scavenging action. Overall, observed positive correlations imply that honey specimens have a considerable antioxidant capacity, which may be verified by several research that were conducted in the past [35,44,46].

3.4. Principal component analysis (PCA)

The PCA was done in this study to have an overview of the similar relationship between antioxidant and physicochemical properties. In order to determine which parameters work best for classifying honey samples, the first four principal components (PCs) that accounted for 98

Table 3
Pearson correlation between physicochemical and antioxidant properties of five Bangladeshi honey samples.

Variables	Moisture	TSS	pH	EC	TDS	*L**	*a**	*b**	HMF	Diastase	Proline	TPC	Flavonoids	DPPH
Moisture	1	−0.178	0.731	−0.855	−0.847	−0.449	0.183	−0.569	.901*	−0.364	−0.498	−0.366	−0.28	.916*
TSS	−0.178	1	−0.138	0.181	0.659	−0.155	0.461	−0.328	−0.424	0.549	0.787	0.26	0.104	−0.551
pH	0.731	−0.138	1	−.911*	−0.54	0.006	0.228	−0.866	0.65	−0.492	−0.606	−.878*	−0.588	0.625
EC	−0.855	0.181	−.911*	1	0.696	0.389	−0.456	0.69	−0.668	0.681	0.7	0.641	0.24	−0.756
TDS	−0.847	0.659	−0.54	0.696	1	0.335	0.101	0.17	−.895*	0.541	0.755	0.298	0.177	.987**
*L**	−0.449	−0.155	0.006	0.389	0.335	1	−0.723	−0.112	−0.107	0.398	0.141	−0.439	−0.713	−0.33
*a**	0.183	0.461	0.228	−0.456	0.101	−0.723	1	−0.246	−0.256	−0.467	−0.084	0.044	0.543	−0.065
*b**	−0.569	−0.328	−0.866	0.69	0.17	−0.112	−0.246	1	−0.456	0.059	0.129	0.751	0.67	−0.309
HMF	.901*	−0.424	0.65	−0.668	−.895*	−0.107	−0.256	−0.456	1	−0.209	−0.511	−0.425	−0.522	.942*
Diastase	−0.364	0.549	−0.492	0.681	0.541	0.398	−0.467	0.059	−0.209	1	.902*	0.4	−0.248	−0.483
Proline	−0.498	0.787	−0.606	0.7	0.755	0.141	−0.084	0.129	−0.511	.902*	1	0.571	0.094	−0.702
TPC	−0.366	0.26	−.878*	0.641	0.298	−0.439	0.044	0.751	−0.425	0.4	0.571	1	0.746	−0.356
Flavonoids	−0.28	0.104	−0.588	0.24	0.177	−0.713	0.543	0.67	−0.522	−0.248	0.094	0.746	1	−0.262
DPPH	.916*	−0.551	0.625	−0.756	−.987**	−0.33	−0.065	−0.309	.942*	−0.483	−0.702	−0.356	−0.262	1

* Correlation is significant at the p < 0.05 level (2-tailed); ** Correlation is significant at the P < 0.01 level (2-tailed).

% of the total variance and had Eigen values less than one were eliminated. The primary significance of the first component was positively correlated with all four of the variables. Therefore, increasing values of EC, TDS, TPC, and flavonoids increased the value of the first principal component. The first principal component is responsible for 59.9 % of the overall data variability, and when combined with the second principal component, it is responsible for 18.3 % of the entire data variability.

In these analyses, EC, TDS, TPC, and flavonoids had significant positive loadings on component 1, so this component measured the high potential of antioxidant activity (Fig. 4). Moisture, pH, and color parameter a* had significant negative loadings on component 2. The negative value of color a* means that this component primarily measured the dark color of honey samples. Moisture does not contribute significantly to component 2, and its impact is lower than other parameters in Component 2. A researcher obtained similar results for Serbian monofloral honey [47].

The score plot (Fig. 4 (b)) indicates an outlier (RH & MH) on the top left, distinguishing from the other samples by significantly higher HMF and DPPH. Furthermore, the right of the top indicates an outlier (LH) denoted from the different samples by significantly higher DN, EC, TPC, L, and color b*. An outlier (SbH) is shown at the bottom of the right portion of the graph. This sample stands out because it has more significant proline, flavonoids, TDS, and TSS levels. On the other hand, the bottom of the left hand represents an outlier (BcH), which can be differentiated from the different samples by having considerably higher

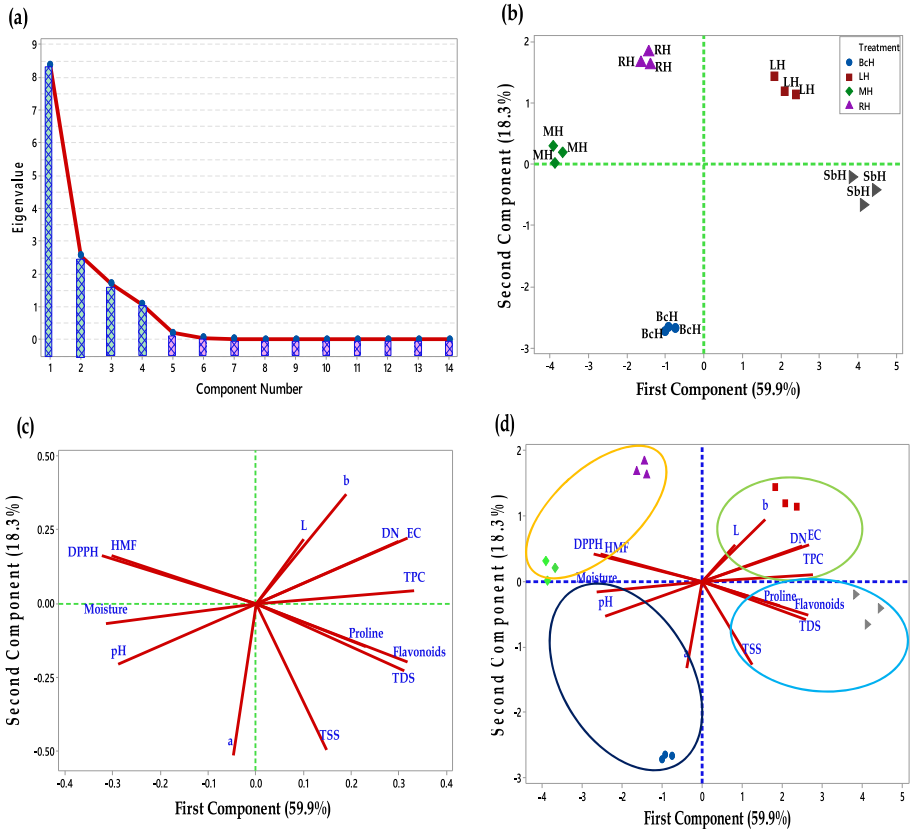

Fig. 4. PCA analysis of five honey samples. (a) The scatter plot depicting the first component in comparison to the second component; (b) comparison of the scores for the first and second components in the score plot; (c) comparison of the first component's loading graphic to the second; (d) Honey's physicochemical and antioxidant characteristics are plotted against one another in a biplot.

moisture levels, color a*, and pH.

The PCA biplots indicated that SbH and LH samples were associated with homogeneity as these are located in the right quadrants. MH, RH, and BcH samples also shared the same homogeneity, similar to other studies under the same circumstances [48]. Thus, these components were employed to analyze five Bangladeshi honey samples for physicochemical and antioxidant homogeneity.

3.5. Hierarchical cluster analysis (HCA)

Cluster Variables were used in this study to group the variables with common characteristics into clusters. The variables were more related to each other with higher similarity levels. When the distance threshold is lowered, the variables are also closer to each cluster. There were a total of 13 different factors at play in these findings. The EC, TDS, HMF, diastase activity, and color (L*, a*, b*) were selected for HCA for their high F-ratio values. Codex Standard for Honey mandates the use of these specific values for moisture and pH. Among honey's antioxidants, TPC, Flavonoids, and DPPH with high F-ratio were retained for HCA analyses.

HCA's resulting dendrogram relied on factors like moisture content, TSS, electrical conductivity, pH, TDS, L*, b*, a*, TPC, Flavonoids, and DPPH (Fig. 5). Firstly, two clusters (variables 4 and 11) were joined, and a new cluster was formed, which created 12 clusters in the data. There was a 99.0484% similarity between the clusters and a 0.01903% dissimilarity. Despite a high degree of resemblance, there were far too many clusters to be useful. Similarity between clusters reduced as new ones arose, until finally all the variables were combined into one. Fig. 5 shows that when the number of clusters was reduced from three to two, there was a sudden fall in similarity at step eleven (85.5708). These findings suggest that a division into three groups is a viable option. High similar characteristics mean a small linkage distance. Cluster analysis was performed on the honey sample data, and three groups were found to have commonalities totaling 61. Therefore, this analysis showed that Cluster 1 (Moisture, pH, HMF, and DPPH), Cluster 2 (EC, TDS, L*, b*, DN, TPC, and Flavonoids), and Cluster 3 (a* and TSS) had high similarity and less distance.

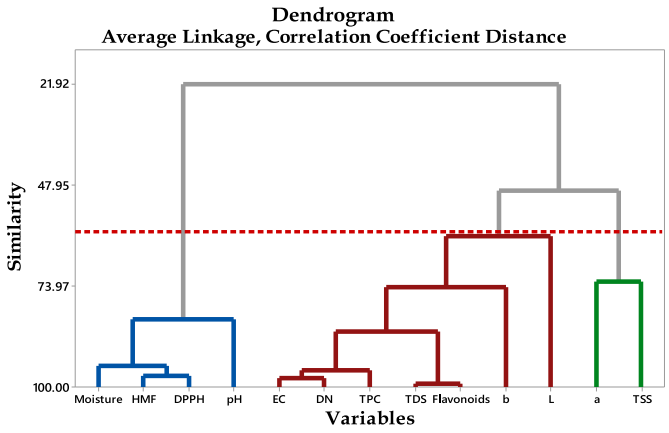

Fig. 5. The similarity-based hierarchical cluster analysis dendrogram.

4. Conclusion

In the current investigation, the physicochemical and antioxidant characteristics of five different samples of Bangladeshi honey were examined. The PCA was done to speculate honey's possible health potential for consumption and business purposes. These honey specimens may be categorized according to their antioxidant capacity as determined by a hierarchical cluster analysis (HCA) for shared physicochemical and antioxidant qualities. The high phenolic, flavonoid levels and lower IC50 values in Bangladeshi honey show that it has retained its antioxidant capabilities. Multi-floral honey contains more phenolics, flavonoids, and DPPH inhibition than mono-floral honey, making it more antioxidant. Sundarbans honey sample fulfilled these criteria as multi-floral honey. The only exception was one sample of Rubber honey with the darkest color but showed lower antioxidant activity than other samples that might have been contaminated during beekeeping. Due to its high moisture content, rubber tree honey cannot be stored for long. It needs to be processed (pasteurized) to prevent fermentation. The present findings provided new constructive information for honey standardization in Bangladesh. All sample findings were within international honey standards, suggesting strong export prospects.

Authors contributions

Sabrina Trisha conducted the laboratory experiments and analyzed the data; Md. Golam Mortuza designed the study and supervised the laboratory work; Juwel Rana assisted in data analysis, graphical abstract, and writing in manuscript preparation; Kazi Hizbul Islam supported in laboratory work and chemical handling; Zannatul Ferdoush assisted in manuscript preparation; Raiya Adiba Antora assisted in writing during manuscript preparation; Sarif Istiak Akash assisted in laboratory work; Mohammad Gulzarul Aziz supervised the study & evaluated the manuscript; M. Burhan Uddin provided language help & proof read the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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