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Determination of Mineral Content and Physico-Chemical Properties of *Apis* Honey from Karnataka State

Dakshayini P N [a], Madhusudan S [b], Bhushanam M [a*], Arun Jyothi Mathias [c] and Abhinandini I D [d]

[a] Department of Zoology, Maharani's Science College for Women, Maharani Cluster University, Bangalore, Karnataka-560 001, India.
[b] Department of Biotechnology, Maharani's Science College for Women, Maharani Cluster University, Bangalore, Karnataka-560 001, India.
[c] Department of Microbiology, Maharani's Science College for Women, Maharani Cluster University, Bangalore, Karnataka-560 001, India.
[d] Department of Zoology, GFGC, Channapatna, Karnataka, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation analyzed *Apis* honey samples of *dorsata, cerana, mellifera* and *florea spp*. from Coorg and Kolar districts of Karnataka. The physical parameters, pH, optical density, viscosity, and moisture contents were analyzed for the *Apis* honey samples and were found to comply with international standards. The efficacy of *Apis* honey in medical research chiefly depends on its mineral constituents. Hence, the total ash content was calculated using the Ivanov and Chevanakova methods. An atomic absorption spectrophotometer and flame photometer were used to quantify minerals in honey samples. The total ash range was between 0.24±0.2 to 0.65±0.1 per

**Corresponding author: Email: mailmadhubiotech@gmail.com;*

cent. The F-test and ANOVA values of the total ash content of honey samples were significant at 5% levels. Honey of *Apis dorsata* had 4.33±0.01 ppm±SD of Calcium and 1.69±0.02 ppm±SD of Magnesium; 0.004±0.0001 ppm±SD of Chromium 0.09±0.02 ppm±SD of Iron was found in honey of *Apis florea*. Likewise, honey of *Apis cerana* had Copper (0.05±0.002 ppm±SD), Manganese (0.08±0.002 ppm±SD), Phosphorus (0.49±0.02 ppm±SD), Potassium (40.44±0.09 ppm±SD) and Sodium (4.37±0.06 ppm±SD). Zinc (0.06±0.002 ppm±SD) was present in honey of *A. mellifera*. All the mineral nutrients studied were within the compliance range and determined to be statistically significant ($p < 0.05$). It was concluded that the Karnataka *Apis* honey maintains its good standards.

Keywords: Apis honey; physico-chemical parameters; mineral content.

1. INTRODUCTION

Honey is a nutritious food with vital complementary elements, including saccharides, organic acids, proteins, minerals, odoriferous substances and limited traces of fats [1]. Using produces nearly 900 tons of honey every year. Its botanical source is the primary factor influencing consumer preference for honey [2]. The mineral nutrients of the honey determine its qualities, enhancing its commercial value, too. Bogdanov *et al.* reported that honey with 0.04 percent mineral content would be light-colored and dark-colored when the mineral content is 0.20 percent.

On the one hand, the healthier benefits of honey are due to trace minerals originating from botanic sources, whereas heavy metals may render the honey toxic [3, 4]. Hence, honey is an ecological marker for various heavy metals, pesticides and environmental contaminants [5]. Therefore, the present investigation focused on assessing a few vital physical parameters and nutraceuticals of *Apis* honey from the Coorg and Kolar regions of Karnataka. It determined the essential micronutrients like Potassium, Phosphorus, Sodium, Manganese, Magnesium, Iron, Copper, Chromium, Calcium and Zinc in the collected *Apis* honey samples. Honey, a nutritious food rich in saccharides, organic acids, proteins, minerals, odoriferous substances, and limited traces of fats, has been documented for its wound healing properties and broad-spectrum antimicrobial effects [6]. Karnataka, producing nearly 900 tons of honey annually, finds its consumer preference influenced primarily by the botanical source of the honey. The mineral content of honey plays a pivotal role in determining its qualities, thereby enhancing its commercial value. For instance, honey with a mineral content of 0.04 percent exhibits a lighter color compared to honey with 0.20 percent mineral content, which appears darker.

2. MATERIALS AND METHODS

Eighty honey samples from various species of Apis bees were procured from different regions of Kolar and Coorg, Karnataka, spanning from 2021 to 2023. The physicochemical parameters of honey were measured using established methods as follows:

1. Optical Density (OD): Measured according to El Sohaimy *et al.* [7].
2. Viscosity: Determined using Ostwald's viscometer based on the Akoh method [8].
3. pH: Measured using a digital pH meter [9].
4. Moisture Content: Assessed using a refractometer [10].
5. Ash Content: Determined using the Ivanov and Chevanakova method [11].
6. Analysis of Minerals or Nutraceuticals in Honey Samples: Each honey sample weighing five grams was digested with nitric perchloric acid in a beaker, filtered, and made up to volume in a 50ml volumetric flask with distilled water. Strontium was added to mitigate interference from phosphorous and aluminum for calcium measurement [12]. Flame photometer was used for measuring Sodium and Potassium, while AAS (Atomic Absorption Spectrometer) was employed for measuring Chromium, Zinc, Copper, Phosphorus, Iron, Calcium, and Manganese [13, 14]. The data on mineral contents were analyzed using *F* test and ANOVA using SPSS.

3. RESULTS AND DISCUSSION

The honey samples collected from different regions of Karnataka exhibited varying pH levels, with Coorg honey samples containing *Apis dorsata* displaying an acidic pH of 5.7±0.1, and *Apis florea* honey showing the lowest pH of 3.7±0.2. In contrast, Kolar honey samples showed a pH range from 5.1±0.4 to 5.9±0.2. The

low pH of *Apis* honey inhibits microbial growth and enhances its texture, stability, and shelf life [15]. Additionally, low pH values contribute to honey's antimicrobial properties [16, 17], making it a desirable alternative to other antimicrobial agents. The Figs. 1 and 2 present the physicochemical characteristics of *Apis* honey samples from Coorg and Kolar regions, respectively. These characteristics include pH levels and optical density, which are indicative of honey's acidity and color, respectively.

The pH of honey samples of *Apis dorsata* and *Apis florea* from Coorg were almost similar to the pH of *Apis florea* and *Apis dorsata* procured from Kolar. The Coorg honey samples containing *Apis dorsata* displayed an acidic pH of 5.7±0.1. In contrast, the *Apis florea* honey showed the least pH of 3.7±0.2. In contrast, in the honey samples from Kolar, the highest pH was recorded for *Apis dorsata* as 5.9±0.2 and the least for *Apis florea* honey at 5.1±0.4 (Fig. 1 & 2). Acidity ranged from 3.86-4.41 in a related study conducted on *A. mellifera* honey samples [18]. *A. mellifera* honey had a mean pH of 3.7 and ranged from 3.4 to 3.9 [19]. The pH values are comparatively high in *Apis* bee honey, 3.75, and 4.21 in Nigerian regions [20]. The study of Siddar honey found that the pH of the tested honey samples stayed between 3.14 and 4.19 [21]. *Apis* honey's low pH significantly reduces the growth and presence of microorganisms [22]. Moreover, this low pH value enhances honey's texture, stability, and shelf life, and *Apis* bee honey plays a crucial role in its extraction and storage [23]. As a possible replacement for other antimicrobial agents, low pH values effectively combat microbes and help prevent some infectious diseases like wounds and coughs [24]. To this fact, *Apis* honey has traditional medicinal value and higher market demand.

The optical density (OD at an absorbance at 560 nm) of *A. dorsata* honey of Coorg was 0.43±0.002, and *A. florea* honey was 0.04±0.01 (Fig.1 & 2). The reason for the increase in OD values could be attributed to crystallisation and granulation of saccharides during storage. At the 0.5% level, the ANOVA for the OD levels of honey produced by the Coorg honey bee species was significant. However, it was not crucial at 0.5% level for *Apis* honey samples from Kolar as it ranged from 18.9±0.2 for *Apis dorsata.* The honey colour in this analysis fell within what White [25] classified as the color of honey. Lighter and fresh kinds of honey possess less OD than stored and dark honey samples. The optical density of *A. cerana* honey varied between 0.29-1.24. The OD of *A. mellifera* honey ranged between 0.33-0.66 [26]. In general, consumers prefer lighter kinds of honey over darker jars of honey.

The viscosity values of the *Apis* honey studied in this work varied between 8.9±0.2 to 12.4±0.6 Ns/m^2 from Coorg and 0.52±0.001 to 13.9±0.8 Ns/m^2 for Kolar honey (Fig. 1 & 2). Similar findings of viscosity range were presented with no more significant difference by Costa *et al.* [27]. The lesser deviation was recorded in honey

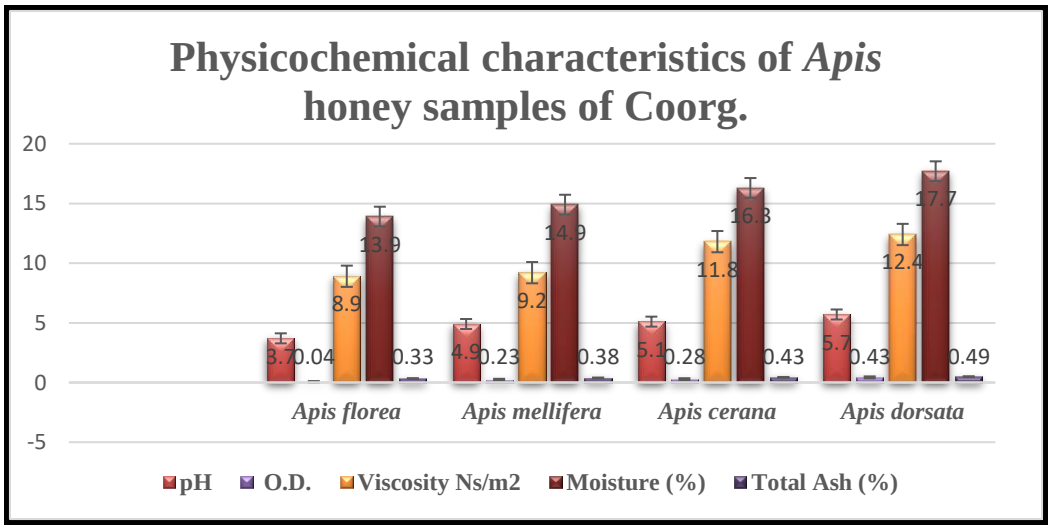

Fig. 1. Physico-chemical characteristics of *Apis* honey samples of Coorg (Mean+SD)

samples harvested as 0.49 to 6.65 in the central region of Rio Grande do Norte, Brazil, by Moreti *et.al.* [28]. A viscosity between 0.36-6.77 was recorded by Sodré *et al.* [29]. The type of nectar defines the viscosity of honey. Honey's high sugar level is primarily responsible for its viscosity. Viscosity is a measurement of a fluid's resistance to flow brought on by internal friction, which causes the liquid to flow at varying rates in different regions. Samples of honey become less dense as the temperature rises. Samples of summer honey were more dense than samples of autumn honey. Increased honey viscosity leads to severe issues during storage, processing and straining.

Due to its high hygroscopic properties, honey quickly absorbs water from its surroundings. As a result, direct air contact will make honey more moisturized. Honey's high moisture content can cause fermentation and hasten the honey's deterioration. The moisture content in honey acceptable by FAO must be <22 %, whereas according to SI (International Standards), the permitted moisture content of honey is <19 %. The *Apis* honey samples from Coorg recorded moisture content ranging from 13.9±0.3to 17.7±0.1% and of Kolar regions from 14.2±0.3 to 17.9%. The lowest range of 13.9% was recorded for *Apis florea s*amples of Coorg and the highest of 17.9±0.2% for *Apis cerana* of Kolar (Fig. 1 & 2). The moisture content of honey samples from Lagos State, Osun State, and Oyo State varies from 14.4% to 17.8% [30]. The average moisture content of the honey samples used in the examination was 16.4%. Following analysis, the moisture contents of raw and fresh honey samples from bee keepers in different Kashmir Valley regions, including pine honeydew, multifloral honey, and Acacia honey, were found to be 18.60, 18.20, and 19.11 percent [31,32]. Samples of multiflora honey collected from viz., Jordan Valley, the Southern Semi-arid region of Palestine, North West Plains, and the Central Mountains, were found to have a moisture content of 16.53 percent. Honey from various Iranian locations and floral varieties ranged from 15.40 to 18.40 percent; multifloral honey had the lowest moisture content. The honey from different regions of eastern Morocco, including rosemary, lavender, eucalyptus, citrus, thyme, carob, multifloral, and jujube, had varying moisture contents:17.92, 18.22, 19.37, 17.76, 16.85, 15.59, 17.17, and 15.39 percent. The moisture content of the honeydew, floral, and fir honeys was 15.20, 17.12 g/100 g [33]. In 2010 and 2013, honey that was collected from local bee keepers in 4 distinct regions of Greece was analyzed, and the results displayed that the moisture content ranged between 15.40-18.59 g/100 g [34]. Subsequently, in 2018, it was reported that honey with varying moisture contents (16.00 to 21.80 percent) was collected from various regions in the Southwest of Kef Tunisia, and included Medicago, Salvia, Rosmarinus, Calluna, Lavandula, Robinia and Citrus [35]. The lack of sugar granules in the samples throughout the various analyses further demonstrated their low moisture content. Despite differences in value, all honey samples from the studied areas fell within the range of internationally acceptable values. Finola *et al.*'s report [36] suggests that the degree of honey maturity in hives before harvest may account for the variation in the obtained values, and it might also refer to the harvest season and storage circumstances used by various farmers in various locations. Honey was found to have comparable moisture contents, according to reports from Kahraman *et al.* [37]. Nonetheless, the study's percentage moisture content was reflected in the samples thickness and flow characteristics, exceeding international standards for acceptability in almost every aspect.

A qualitative relationship exists between the varied honey's botanical regions and the ash content variability. It is a crucial requirement to identify the honey's botanical origin potentially. Following honey carbonization, the amount of ash in the honey directly indicates the inorganic residues. The floral sources of honey account for the variation in ash content of honey samples from the areas under analysis. Every honey sample examined in this study fell within Bogdanov's suggested range (<0.65%) [38]. The range recorded was between 0.24±0.2 to 0.65±0.1%. The lowest range of 0.24±0.2% was analyzed for *Apis florea* samples of Kolar, and the highest was 0.65% of *Apis mellifera*of Kolar (Fig. 1 & 2). Studies by Kahraman et al. [28] and Adenekan *et al*. reported ash contents comparable to those in the present study. An approximate finding was recorded by Great Iruoghene Edo *et al.* [39] in fresh honey samples (0.58 ± 0.15%). With an average value of percent the honey samples' ash content ranged from 0.014 to 0.31g [40]. All the examined honey samples had ash contents between 0.01 and 1.2%, per the Quality and Standards Authority of Ethiopia (QSAE) [41]

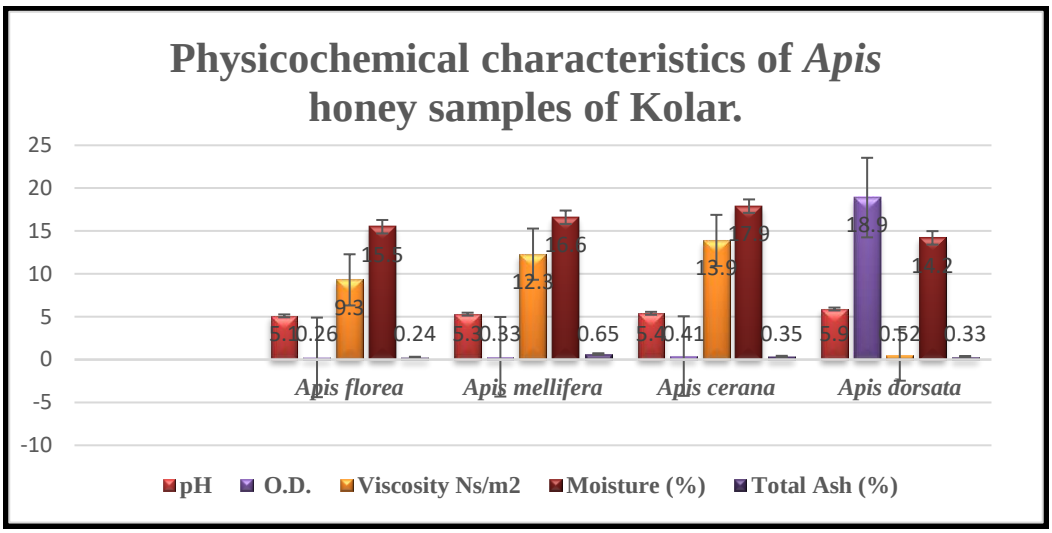

Fig. 2. Physico-chemical characteristics of *Apis* honey samples of Kolar (Mean+SD)

Table 1. Nutraceutical constituents of *Apis* honey samples from Coorg, Karnataka during 2021-23

Honey Samples	Mineral Type *(ppm±S.D)*									
	Ca	Cr	Cu	Fe	Mg	Mn	P	K	Na	Zn
Apis	4.22±	0.004±	0.03±	0.09±	1.02±	0.08±	0.49±	40.01±	4.22±	0.06±
florea	0.01	0.0001	0.002	0.02	0.03	0.004	0.03	0.04	0.03	0.003
Apis	3.63±	0.002±	0.05±	0.06±	1.44±	0.08±	0.49±	40.44±	4.37±	0.04±
cerana	0.02	0.0006	0.002	0.02	0.02	0.002	0.02	0.09	0.06	0.003
Apis	4.21±	0.002±	0.04±	0.05±	1.58±	0.06±	0.51±	40.11±	4.16±	0.06±
mellifera	0.01	0.0008	0.006	0.02	0.06	0.007	0.08	0.04	0.09	0.002
Apis	4.33±	0.001±	0.04±	0.04±	1.69±	0.04±	0.53±	40.08±	4.11±	0.06±
dorsata	0.01	0.0002	0.003	0.02	0.02	0.009	0.07	0.06	0.05	0.008

Significant at p< 0.05 levels

Table 2. Nutraceutical constituents of *Apis* honey samples from Kolar, Karnataka, during 2021-23

Honey Samples	Mineral Type *(ppm±S.D)*									
	Ca	Cr	Cu	Fe	Mg	Mn	P	K	Na	Zn
Apis	3.33±	0.003±	0.02±	0.05±	0.7±	0.06±	0.37±	36.01±	3.91±	0.03±
florea	0.05	0.0001	0.007	0.01	0.001	0.002	0.01	0.02	0.01	0.002
Apis	3.12±	0.003±	0.03±	0.04±	0.6±	0.06±	0.33±	33.32±	3.90±	0.03±
cerana	0.01	0.0002	0.006	0.06	0.003	0.001	0.05	0.01	0.02	0.005
Apis	3.11±	0.003±	0.03±	0.04±	0.6±	0.05±	0.31±	30.5±	3.88±	0.04±
mellifera	0.03	0.0007	0.002	0.01	0.009	0.009	0.02	0.03	0.01	0.001
Apis	3.10±	0.002±	0.03±	0.03±	0.5±	0.05±	0.30±	30.6±	3.79±	0.04±
dorsata	0.04	0.0001	0.001	0.01	0.004	0.003	0.01	0.02	0.01	0.003

Significant at p< 0.05 levels

Higher amounts of Sodium, Phosphorus, Magnesium, Calcium, and Potassium were documented in *Apis* honey samples than other minerals examined in this study. Honey of *Apis dorsata* had 4.33±0.01 ppm±SD of Calcium and 1.69±0.02 ppm±SD of Magnesium; 0.004±0.0001 ppm±SD of and Chromium 0.09±0.02 ppm±SD of Iron was found in honey of *Apis florea*. Likewise, honey of *Apis cerana* had Copper (0.05±0.002 ppm±SD), Manganese (0.08±0.002 ppm±SD), Phosphorus (0.49±0.02 ppm±SD), Potassium (40.44±0.09 ppm±SD) and Sodium (4.37±0.06 ppm±SD). Zinc (0.06±0.002 ppm±SD) was present in honey of *Apis mellifera.* .All the

mineral constituents analyzed were well within the range and determined to be statistically significant $p<0.05$ levels (Table 1 & 2). Also, the mineral contents were recorded to be comparatively higher for *Apis* samples of Coorg than Kolar regions. The mineral composition of *Apis* honey samples reveals higher amounts of essential nutrients such as Calcium, Potassium, and Magnesium compared to other minerals. These minerals are vital for various biochemical processes and may serve as indicators of the honey's geographical origin [42, 43]. The presence of minerals in honey can originate from both natural sources such as plants and soil, as well as anthropogenic sources like pollutants. Dark-colored honey samples, such as those from *Apis cerana* honey of Shimla, are reported to contain more minerals and exhibit high medicinal and nutritional value [44] which is in accordance with our findings. Similar outcomes were described by Tuzen *et al.* [45] in honey from Turkey and other nations like Italy and Spain. Rashed and Soltan [46] reported increased values for these components from Egyptian honey samples. Potassium was the most abundant of the elements determined, with an average content of 1572 mg/kg (38.5% of the ash). All mineral contents showed high coefficients of variation, ranging from 0.34 (Sodium, Calcium, and Sulphur) to 0.71 (Iron). In addition to being necessary for human health, these minerals may be crucial for several biochemical processes. Since the differences in mineral elements between honey samples from various nations are widely accepted, numerous studies have noted that minerals can serve as environmental indicators as well as be a strong indicator of the honey's geographical origin. During their foraging activity, honeybees may continuously be exposed to pollutants in the surrounding area [47]. According to reports [48, 49], honey minerals may come from anthropogenic and natural sources, such as plants and soil. The highest average Chromium values they recorded in the city samples, 0.010 mg/kg, followed by the village samples with 0.006 mg/kg, and the rural and the mountain samples with an average of 0.004 mg/kg. The average Iron. values in the sample from mountain, rural, city and village area recorded were 0.73, 1.34, 1.70 and 1.80 mg/kg. Not only are minerals like Iron and Manganese naturally occurring in soil minerals but they are also recognized as possibly anthropogenic air and soil contaminants like Zinc and Copper. According to Bogdanov *et al.* , honey minerals come from anthropogenic and natural sources, such as plants and soil. In addition to being directly excreted as nectar, these substances may also be found on leaf surfaces [50], in honeydew, on bees, or by being deposited as specks of dust or aerosols on flowers and nectars. The *Apis cerana* honey samples of Shimla were reported to have a dark color, and showed more minerals than light colored honey. This honey has proven to have high medicinal and nutritional value [51,52].

4. CONCLUSION

The *Apis* honey samples assessed were from the Coorg and Kolar regions of Karnataka, India. The physical parameters that were analyzed fell between the BIS and international standards. The present study provides a comprehensive data set on comparative elemental nutraceutical levels in *Apis* honey. The results of this investigation reveal the quality of Indian honey remains good (Honey of Coorg is better than Kolar) concerning the concentration of minerals and provide a safety base line levels for human consumption of honey products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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