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# Physical properties of the stingless bee honey in comparison with *Apis cerana* honey samples collected from different geographical locations of Karnataka and Kerala

**Moulya MR, Jagadish KS, Anilkumar ST, Priyanka and Eswarappa G**

**Abstract**
The physical parameters like pH, electrical conductivity, specific gravity, colour, optical density, refractive index, moisture content, total solids, total soluble solids and ash content were analyzed in all stingless bee honey and *A. cerana* honey samples collected from different geographical locations of Karnataka and Kerala. The pH, EC, colour, optical density, moisture and ash content was higher in stingless bee honey than the *A. cerana* honey with the mean values of about 4.84, 1.32 dS/m, 150 mm pfund value, 1.231, 23.34% and 0.172%, respectively. The remaining parameters like specific gravity, refractive index @ 20 °C, total solids and total soluble soilids were highest in *A. cerana* honey than the stingless bee honey with the mean value of about 1.43, 1.484, 79.89% and 76.17%, respectively. The floral resources and geographic origins of the honey can result in significant variations of honey quality parameters.

**Keywords:** *Apis cerana*, stingless bee, honey, physical parameters

## Introduction
Honey is known as "Nature's Ointment" because honeybees collect nectar from flowers and convert it into a product that is a healthier alternative to cane sugar. Honey is a semi-liquid substance made up of a complex mixture of sugars and water as the primary ingredients. A slew of other constituents that gives unique characteristics of honey such as taste and flavour. Enzymes, vitamins, minerals, pigments, natural physiologically active chemicals, organic acids and various lactones are the most significant components. People have been consuming it as the most delicious and trustworthy sweetener without processing for a long time, according to evidence from civilization remnants (Da Silva *et al.*, 2016) [1]. The floral source, geographic variables (seasonal and environmental factors) and processing techniques all have an impact on the composition of honey (Alvarez-Suarez *et al.*, 2010) [2]. Stingless bee honey is an important product that has been used for centuries by indigenous communities in tropical regions of the world for its medicinal and nutritional properties. Stingless bee honey has been shown to have a range of medicinal properties, including antibacterial, anti-inflammatory, antioxidant, and antifungal effects. It is traditionally used to treat a variety of ailments, including respiratory problems, skin infections, digestive disorders and even cancer. Overall, stingless bee honey is an important product that provides both nutritional and medicinal benefits, while also being sustainable and environmental friendly. The physicochemical features of stingless bee honey are not well explored. As a result, the current study's goal is to compare the physical features of stingless bee honey to honey from *Apis cerana*.

## Material and Methods
The physical properties such as pH, electrical conductivity (dS/m), specific gravity, colour-pfund values (mm), optical density, refractive index, moisture content (%), total solids (%), total soluble solids (˚Brix) and ash content (%) of stingless bee honey and *A. cerana* honey collected from twelve different locations of Karnataka (nine) and Kerala (three) were determined at the post graduate laboratory, Department of Apiculture and Department of Plant Biotechnology, UAS, GKVK, Bengaluru. A total of 24 samples (twelve each of *Tetragonula* sp. and *Apis cerana*) were examined. Places like Kannur, Muliyar, Badiyadka, Shivamogga, Chikkamagaluru, Ponnampet, Ballari, Raichur, Panaje, Darbe, GKVK and Sullia were the locations chosen for the collection of both stingless bee honey and *Apis cerana* honey.

**pH**
A representative sample of 10 gram of honey was drawn from each replication of honey samples collected from the specific locations and were dissolved in 50 ml of distilled water. The pH was recorded directly from the pH meter.

**Specific gravity**
Specific gravity of each representative sample of honey was estimated by using a specific gravity bottle and thermostatically controlled water-bath, which was maintained at 27±1 °C. The empty specific gravity bottle was cleaned, dried thoroughly and weighed (A). After that, the distilled water was filled, which was maintained at 27±1 °C upto the mark and weighed (B). Further, the water was removed from the bottle, dried and refilled with the collected honey sample upto the mark and its weight was recorded (C). The specific gravity of the honey sample was calculated by using the following formula as per the methodology suggested by Anon. (1974) [3].

$$\text{Specific gravity at 27 °C} = \frac{C - A}{B - A}$$

A = Mass of empty specific gravity bottle (g)
B = Mass of specific gravity bottle with water (g)
C = Mass of Specific gravity bottle with honey sample (g)

**Electrical conductivity (EC)**
Ten gram of each honey samples was dissolved thoroughly in a beaker having 25 ml of distilled water and fed to EC cells of electric conductivity apparatus. The readings were expressed as deci-Siemens per meter (Anon., 1974) [3].

**Pfund - Colour**
The colour of each representative sample of honey was recorded by using honey colour analyser (Hanna instruments) based on pfund scale readings.

**Ash content**
The ash content in the different honey samples was estimated by burning 10 g of each honey sample with a few drops of pure olive oil in a silica or platinum dish and then it was ignited in a Muffle furnace @ 600±20 °C till white ash was obtained and finally the contents were weighed (Anon., 1990) [4].

$$\text{Ash percent by mass} = \frac{100\ (M_2 - M_1)}{(M_1 - M)}$$

M = Mass (g) of empty dish
$M_1$ = Mass (g) of the dish with material taken for test
$M_2$ = Mass (g) of the dish with ash

**Moisture Content**
The moisture content of collected honey samples was determined by using a refractometer.

**Optical Density**
Two gram of each representative honey sample was weighed in a small beaker and dissolved in distilled water. The volume was made upto 10 ml by using a 10 ml measuring cylinder. The colorimeter was adjusted with distilled water in a cuvet at '0' absorbance or 100 per cent transmittance at 600 nm. The honey solution was taken in a cuvet and the absorbance was read directly as the per cent transmittance at the same wavelength (Anon., 1974) [3].

Optical density = 2 - log per cent transmittance

**Refractive index**
The refractive index of honey samples was determined by using Abbe refractometer at ambient temperature.

**Total soluble solids (°Brix)**
Total soluble solids (TSS) of the different honey samples were directly measured by using a hand refractometer and the results were expressed in °Brix.

**Total solids (%)**
The total solids content (TS) of the different honey samples were determined by using the formula given by Anon. (1990) [4] and it was expressed in percentage.

TS (%) = 100 - moisture content

**Results**
The physical parameters of total 24 honey samples (twelve each of *Tetragonula* spp. and *Apis cerana*) were examined (Table1& 2) (Fig. 1, 1a, 2 & 2a).

**Table 1:** Physical parameters of stingless bee honey and *Apis cerana* honey collected from different parts of Karnataka and Kerala during 2021-22

| Locations | pH | | EC | | Specific gravity | | pfund values | | Refractive index @ 20 °C | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SBH | ACH | SBH | ACH | SBH | ACH | SBH | ACH | SBH | ACH |
| **A) Kerala** | | | | | | | | | | |
| Kannur | 4.74$^{g}$ | 5.21$^{a}$ | 0.42$^{j}$ | 0.80$^{j}$ | 1.457$^{b}$ | 1.393$^{h}$ | 114.33$^{b}$ | 56.67$^{f}$ | 1.482$^{ab}$ | 1.483$^{abc}$ |
| Muliyar | 4.85$^{c}$ | 5.06$^{c}$ | 2.39$^{a}$ | 1.32$^{d}$ | 1.411$^{e}$ | 1.441$^{d}$ | 101.33$^{c}$ | 43.00$^{j}$ | 1.472$^{ab}$ | 1.474$^{d}$ |
| Badiyadka, Kasargodu | 4.84$^{d}$ | 4.02$^{i}$ | 0.51$^{i}$ | 1.21$^{e}$ | 1.422$^{d}$ | 1.438$^{d}$ | 150.00$^{a}$ | 108.00$^{b}$ | 1.478$^{ab}$ | 1.480$^{bc}$ |
| B) Karnataka | | | | | | | | | | |
| Shivamogga | 4.85$^{c}$ | 4.28$^{h}$ | 1.16$^{e}$ | 1.94$^{b}$ | 1.438$^{c}$ | 1.431$^{e}$ | 150.00$^{a}$ | 53.00$^{g}$ | 1.466$^{b}$ | 1.483$^{bc}$ |
| Ponnampet, Kodagu | 5.01$^{a}$ | 5.12$^{b}$ | 2.34$^{b}$ | 1.45$^{c}$ | 1.383$^{g}$ | 1.458$^{c}$ | 150.00$^{a}$ | 59.67$^{e}$ | 1.477$^{ab}$ | 1.485$^{ab}$ |
| Chikkamagaluru | 4.72$^{i}$ | 4.98$^{d}$ | 0.93$^{f}$ | 1.12$^{f}$ | 1.382$^{g}$ | 1.350$^{i}$ | 150.00$^{a}$ | 48.00$^{h}$ | 1.488$^{a}$ | 1.487$^{a}$ |
| Ballari | 4.91$^{b}$ | 4.01$^{i}$ | 0.65$^{g}$ | 0.89$^{i}$ | 1.304$^{j}$ | 1.494$^{a}$ | 150.00$^{a}$ | 32.67$^{k}$ | 1.468$^{b}$ | 1.487$^{a}$ |
| Raichur | 4.84$^{d}$ | 3.95$^{j}$ | 1.26$^{d}$ | 0.91$^{i}$ | 1.370$^{h}$ | 1.423$^{f}$ | 150.00$^{a}$ | 45.00$^{i}$ | 1.474$^{ab}$ | 1.478$^{cd}$ |
| Panaje, Dakshina Kannada | 4.73$^{h}$ | 4.57$^{g}$ | 0.56$^{h}$ | 0.99$^{g}$ | 1.469$^{a}$ | 1.412$^{g}$ | 150.00$^{a}$ | 87.67$^{c}$ | 1.490$^{a}$ | 1.488$^{a}$ |
| Darbe, Dakshina Kannada | 4.83$^{e}$ | 4.80$^{f}$ | 2.05$^{c}$ | 0.95$^{h}$ | 1.352$^{i}$ | 1.481$^{b}$ | 150.00$^{a}$ | 150.00$^{a}$ | 1.473$^{ab}$ | 1.489$^{a}$ |
| Sullia, Dakshina Kannada | 4.81$^{f}$ | 4.82$^{f}$ | 1.25$^{d}$ | 1.01$^{g}$ | 1.412$^{e}$ | 1.423$^{f}$ | 150.00$^{a}$ | 88.00$^{c}$ | 1.247$^{c}$ | 1.492$^{a}$ |
| GKVK, Bengaluru | 4.91$^{b}$ | 4.90$^{e}$ | 2.32$^{b}$ | 2.74$^{a}$ | 1.394$^{f}$ | 1.411$^{g}$ | 150.00$^{a}$ | 66.00$^{d}$ | 1.473$^{ab}$ | 1.479$^{cd}$ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 4.84 | 4.64 | 1.32 | 1.28 | 1.400 | 1.430 | 142.97 | 69.81 | 1.457 | 1.484 |
| S.D. | 0.004 | 0.007 | 0.022 | 0.011 | 0.002 | 0.003 | 0.197 | 0.331 | 0.005 | 0.001 |
| SEm± | 0.003 | 0.005 | 0.016 | 0.008 | 0.001 | 0.002 | 0.139 | 0.234 | 0.004 | 0.001 |
| CD @ p=0.01 | 0.009 | 0.014 | 0.047 | 0.023 | 0.003 | 0.006 | 0.411 | 0.690 | 0.011 | 0.002 |

* Values in the column followed by common letters are not significantly different at p = 0.01 as per Tukey"s HSD (Tukey, 1965);

**Table 1a:** Physical parameters of stingless bee honey and *Apis cerana* honey collected from different parts of Karnataka and Kerala during 2021-22

| Locations | Optical density | | Moisture content (%) | | Total solids (%) | | Total soluble solids (°Brix) | | Ash content (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SBH | ACH | SBH | ACH | SBH | ACH | SBH | ACH | SBH | ACH |
| **A) Kerala** | | | | | | | | | | |
| Kannur | 0.513$^{k}$ | 0.337$^{b}$ | 21.67$^{de}$ | 20.23$^{c}$ | 79.20$^{c}$ | 79.47$^{d}$ | 75.83$^{a}$ | 75.50$^{cd}$ | 0.086$^{efg}$ | 0.139$^{d}$ |
| Muliyar | 0.280$^{l}$ | 0.150$^{de}$ | 24.85$^{abc}$ | 23.80$^{a}$ | 76.37$^{def}$ | 76.60$^{g}$ | 74.80$^{b}$ | 74.33$^{e}$ | 0.096$^{defg}$ | 0.065$^{j}$ |
| Badiyadka, Kasargodu | 0.740$^{i}$ | 0.287$^{bc}$ | 21.93$^{de}$ | 19.20$^{cd}$ | 77.13$^{d}$ | 78.40$^{e}$ | 74.00$^{bc}$ | 76.16$^{b}$ | 0.147$^{de}$ | 0.164$^{b}$ |
| B) Karnataka | | | | | | | | | | |
| Shivamogga | 0.887$^{g}$ | 0.187$^{cde}$ | 25.60$^{ab}$ | 19.33$^{de}$ | 76.20$^{ef}$ | 80.33$^{c}$ | 73.80$^{c}$ | 76.33$^{b}$ | 0.035$^{g}$ | 0.142$^{d}$ |
| Ponnampet, Kodagu | 2.977$^{a}$ | 0.123$^{de}$ | 23.53$^{bcd}$ | 19.34$^{cd}$ | 76.53$^{de}$ | 81.13$^{b}$ | 74.01$^{bc}$ | 76.50$^{b}$ | 0.545$^{a}$ | 0.194$^{a}$ |
| Chikkamagaluru | 0.767$^{h}$ | 0.110$^{de}$ | 20.27$^{e}$ | 19.25$^{de}$ | 81.40$^{b}$ | 81.20$^{b}$ | 73.33$^{c}$ | 78.50$^{a}$ | 0.134$^{def}$ | 0.113$^{g}$ |
| Ballari | 1.387$^{d}$ | 0.110$^{de}$ | 26.73$^{a}$ | 19.47$^{cd}$ | 73.27$^{h}$ | 81.27$^{b}$ | 69.17$^{e}$ | 76.17$^{b}$ | 0.236$^{b}$ | 0.121$^{ef}$ |
| Raichur | 2.923$^{b}$ | 0.137$^{de}$ | 24.47$^{bc}$ | 22.87$^{b}$ | 75.60$^{fg}$ | 77.47$^{f}$ | 70.17$^{d}$ | 75.17$^{d}$ | 0.222$^{bc}$ | 0.150$^{c}$ |
| Panaje, Dakshina Kannada | 1.067$^{f}$ | 0.230$^{bcd}$ | 18.67$^{f}$ | 18.60$^{de}$ | 82.33$^{a}$ | 81.47$^{ab}$ | 76.17$^{a}$ | 78.00$^{a}$ | 0.167$^{cd}$ | 0.093$^{i}$ |
| Darbe, Dakshina Kannada | 1.440$^{c}$ | 0.490$^{a}$ | 24.87$^{abc}$ | 18.20$^{e}$ | 75.13$^{g}$ | 81.93$^{a}$ | 70.15$^{d}$ | 75.83$^{bc}$ | 0.082$^{fg}$ | 0.102$^{h}$ |
| Sullia, Dakshina Kannada | 1.233$^{e}$ | 0.323$^{b}$ | 23.30$^{cd}$ | 19.03$^{de}$ | 76.43$^{de}$ | 81.50$^{ab}$ | 73.43$^{c}$ | 76.23$^{b}$ | 0.092$^{efg}$ | 0.121$^{f}$ |
| GKVK, Bengaluru | 0.553$^{j}$ | 0.100$^{e}$ | 24.20$^{bc}$ | 21.60$^{b}$ | 75.80$^{efg}$ | 77.93$^{ef}$ | 73.27$^{c}$ | 75.33$^{cd}$ | 0.227$^{bc}$ | 0.125$^{e}$ |
| Mean | 1.231 | 0.215 | 23.34 | 20.07 | 77.12 | 79.89 | 73.18 | 76.17 | 0.172 | 0.127 |
| S.D. | 0.005 | 0.033 | 0.572 | 0.261 | 0.326 | 0.263 | 0.362 | 0.283 | 0.027 | 0.002 |
| SEm± | 0.003 | 0.023 | 0.404 | 0.184 | 0.231 | 0.186 | 0.256 | 0.200 | 0.019 | 0.001 |
| CD @ p=0.01 | 0.010 | 0.069 | 1.193 | 0.544 | 0.680 | 0.549 | 0.755 | 0.591 | 0.057 | 0.003 |

* Values in the column followed by common letters are not significantly different at p = 0.01 as per Tukey"s HSD (Tukey, 1965);

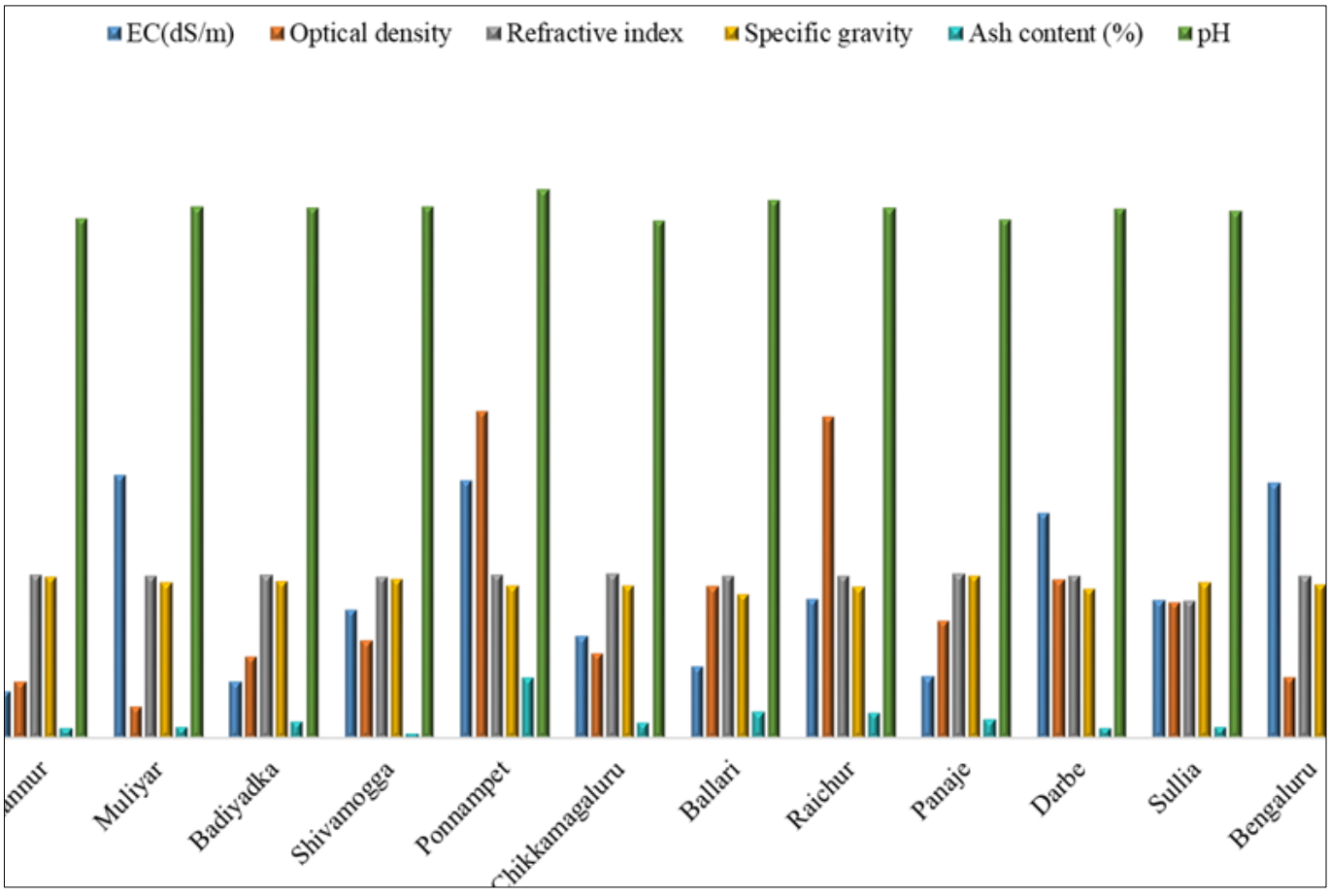


**Fig 1:** Physical parameters of Stingless bee honey collected from different locations of Karnataka and Kerala

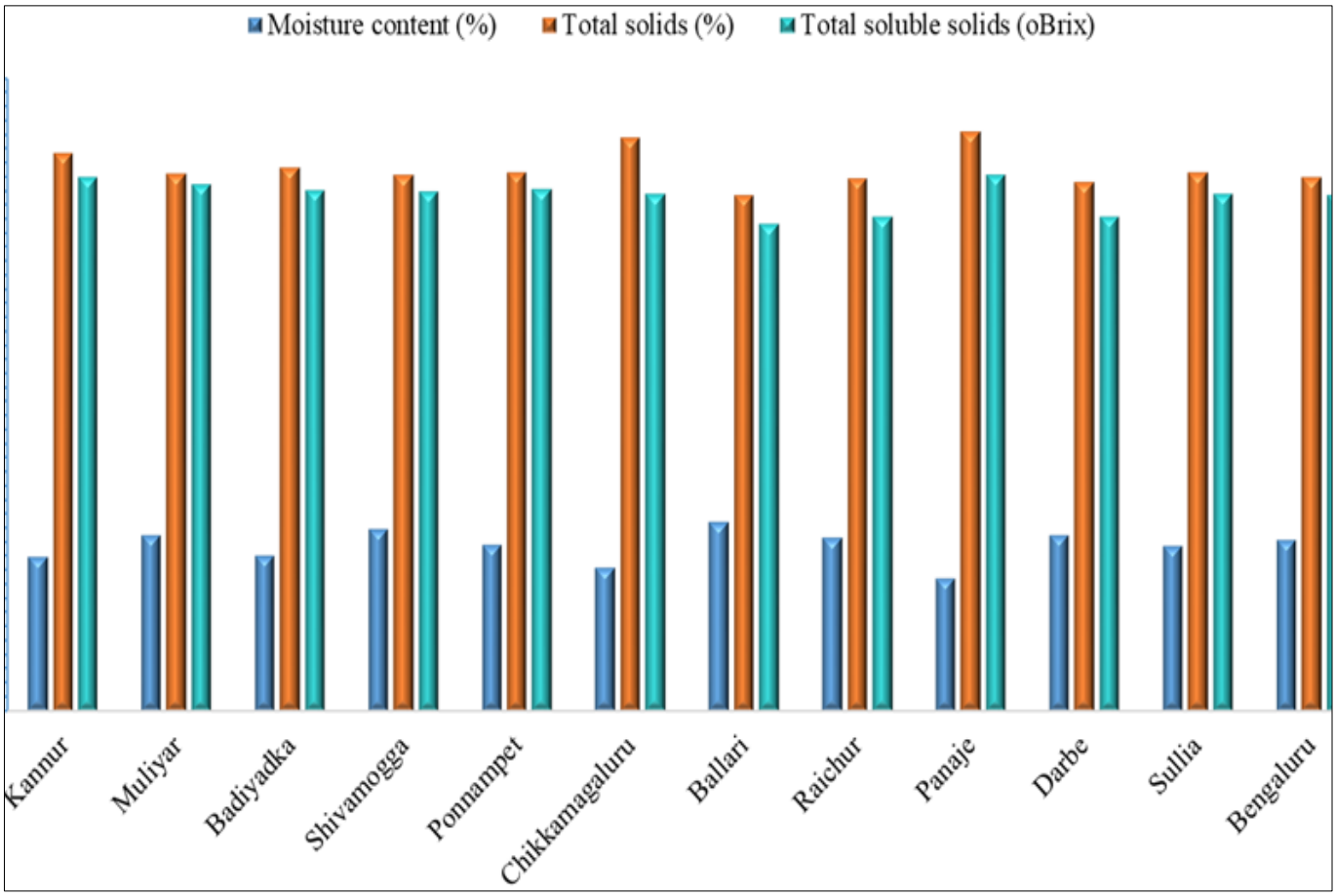


**Fig 1a:** Physical parameters of Stingless bee honey collected from different locations of Karnataka and Kerala

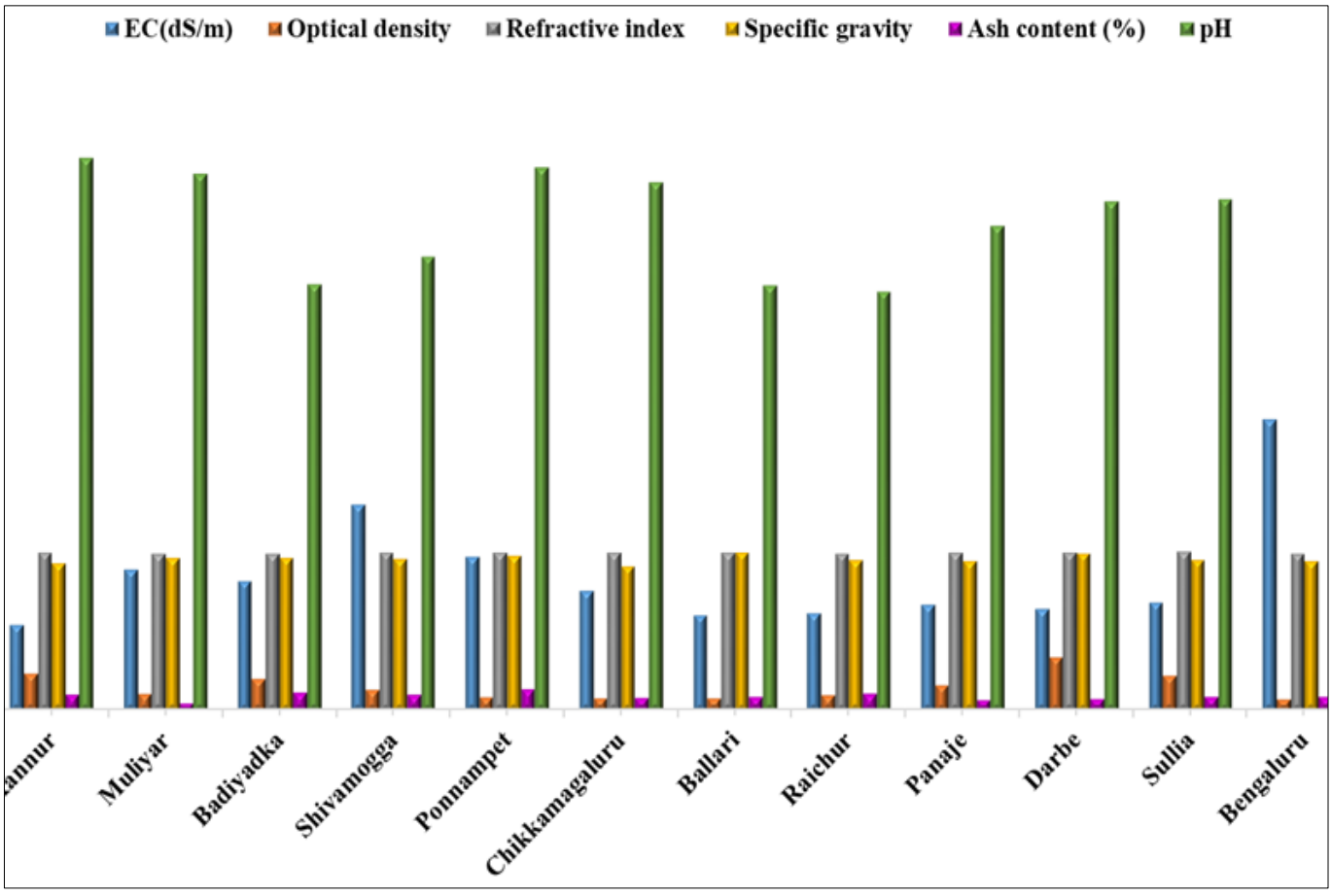


**Fig 2:** Physical parameters of *Apis cerana* honey collected from different locations of Karnataka and Kerala

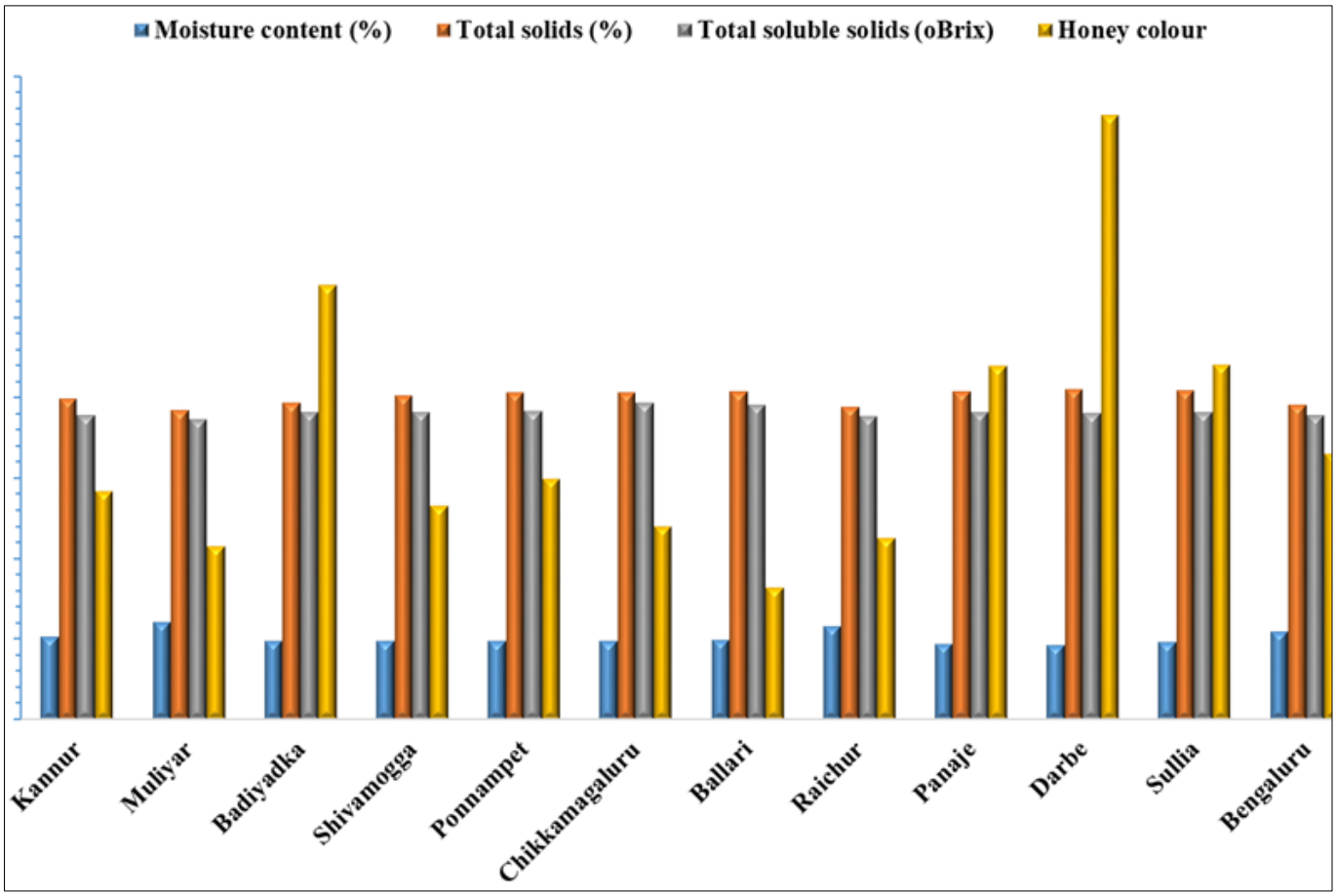


**Fig 2a:** Physical parameters of *Apis cerana* honey collected from different locations of Karnataka and Kerala

**pH**

Honey is naturally acidic due to organic acids and its pH varies based on factors such as origin, soil conditions and mineral content. In a study, stingless bee honey from Ponnampet had the highest pH (5.01) and *A. cerana* honey from Kannur had the highest pH (5.21). The mean pH of *A. cerana* honey (4.64) was lower than that of stingless bee honey (4.84). Variations in pH can affect honey's physico-chemical properties and preservation against microbial spoilage. Hence, analyzing honey pH is essential to evaluate its quality and safety.

**EC**

The electrical conductivity of honey is related to its concentration of mineral salts, organic acids, and proteins and is useful for determining its floral origin and purity. Stingless bee honey had an electrical conductivity range of 0.42-2.39 dS/m (1.32 dS/m), with the highest values in Muliyar (2.39 dS/m), Ponnampet (2.34 dS/m), and GKVK (2.32 dS/m) samples. *A. cerana* honey had an electrical conductivity range of 0.80-2.74 dS/m (1.28 dS/m), with the highest values in GKVK (2.74 dS/m), Shivamogga (1.94 dS/m) and Ponnampet (1.45 dS/m) samples. The lowest electrical conductivity value was recorded in Kannur honey for both stingless bee (0.42 dS/m) and *A. cerana* honey (0.80 dS/m).

**Specific gravity**

The specific gravity of honey is an important physical characteristic that depends on its water content and is used to express its density. Stingless bee honey had a specific gravity range of 1.304-1.469 (1.400), while *A. cerana* honey had a range of 1.350-1.494 (1.430). The maximum specific gravity of stingless bee honey was 1.469 in Panaje, while the maximum for *A. cerana* honey was 1.494 in Ballari. The minimum specific gravity for Kannur and Chikkamagaluru honey was 1.393 and 1.350, respectively.

**pfund values (Colour)**

The color of honey is an important characteristic that contributes to its appearance. Stingless bee honey is generally darker in color compared to *A. cerana* honey. The pfund values of stingless bee honey ranged from 101.33 mm to 150.00 mm with the mean value of 142.97 mm, with most samples being classified as "Dark amber". The pfund values of *A. cerana* honey ranged from 32.67 mm to 150.00 mm with the mean value of 69.81 mm, with some samples being classified as "Light amber" and others as "Extra light amber".

**Optical density**

Optical density is an important factor in determining the colour and freshness of honey. In this study, the optical density of stingless bee honey ranged from 0.513 - 2.977 (1.231), with Ponnampet honey having the highest optical density of 2.977, followed by Raichur, Darbe, Ballari, and Sullia. The optical density of *A. cerana* honey ranged from 0.100 - 0.490 (0.215), with Darbe honey having the highest optical density. The optical density values of Panaje, Shivamogga, Muliyar, Raichur, Ponnampet, Ballari and Chikkamagaluru were statistically similar. GKVK honey had the lowest optical density. Overall, optical density values can provide important information about the colour and freshness of honey.

**Refractive index**
Refractive index is an important characteristic of honey and can be used to determine its moisture level. The refractive index of stingless bee honey ranged from 1.247 – 1.490 (1.457), with Panaje and Chikkamagaluru honey having the highest values. The refractive index of *A. cerana* honey ranged from 1.474 – 1.492 (1.484), with Sullia honey having the highest value.

**Moisture content**
Moisture content is an important factor that affects honey quality. The moisture content of stingless bee honey ranged from 18.67 - 26.73% (23.34%), with Ballari honey having the highest moisture content and Panaje sample having the lowest. The moisture content of *A. cerana* honey ranged from 18.20 - 23.80% (20.07%), with Muliyar honey having the highest moisture content and Darbe honey having the lowest.

**Total solids**
Total solids in honey measure the combined contents of all inorganic and organic substances. It can be used to determine the purity and cleanliness of honey. The total solids of stingless bee honey ranged from 73.27% to 82.33%, with the highest values found in Panaje and Chikkamagaluru. The total solids of *A. cerana* honey ranged from 76.60% to 81.93%, with the highest values found in Darbe and Sullia.

**Total soluble solids (TSS)**
The total soluble solids of honey vary depending on the types and amounts of sugars present, which are influenced by the source of the nectar. The total soluble solids of stingless bee honey ranged from 69.17% to 76.17% with the mean value of 77.12% and highest TSS found in Panaje and Kannur samples. The total soluble solids of *A. cerana* honey ranged from 74.33% to 78.50% with the mean value of 73.18% and highest levels found in Chikkamagaluru and Panaje samples. Soluble solids in honey include sugars, organic acids, and minerals.

**Ash content**
The ash content of honey is an important factor that affects its quality. The ash content of stingless bee honey ranged from 0.227% to 0.545% (0.172%), with Ponnampet having the highest ash content and Shivamogga having the lowest. The ash content of *A. cerana* honey ranged from 0.065% to 0.194% (0.127%), with Ponnampet having the highest and Muliyar having the lowest.

**Discussion**
The results of the present investigations were supported by Anusha *et al.* (2020) [5], the pH of stingless bee honey ranged from 3.7±0.00 to 5.92±0.02 whereas the pH of *A. cerana* honey samples ranged from 3.00±0.02 to 4.67±0.02. The electrical conductivity (mS/cm) of stingless bee honey was 0.94±0.01 to 2.51±0.06 and honey from *Apis* sp. ranged between 0.16±0.00 to 1.08±0.00. The moisture content (%) of stingless bee honey was 15.0±0.06 to 30.0±0.06 whereas the *Apis* honey had moisture content from 18.10±0.15 to 40.30±0.20. Since the nectar's pH and the soil's conditions have a significant impact on the honey's physico-chemical properties, the floral and geographic origins of the honey can result in significant pH variations (Wang, 2011) [6]. Biluca *et al.* (2016) [15], Nweze *et al.* (2017) [8] and Cardona *et al.* (2019) [9] who reported that stingless bee honey has higher moisture content as compared to *A. cerana* honey. According to Hanson (1951) [10], honey is both hygroscopic and hygrophobic, and its water content rises as relative humidity rises. Honey begins to ferment as its moisture level rises. Honey contains sugars, organic acids, and minerals as soluble solids. The value of total soluble solids parameter illustrates the connection between the water and sugar content (Biluca *et al.*, 2016) [15]. Because stingless bee honey contains more water and less sugar than *Apis cerana* honey. Nordin *et al.* (2018) [11] and Asaduzzaman *et al.* (2015) [12] who reported that the ash content is highest in stingless bee honey as compared to others. The mineral concentration of honey is correlated with its ash level, which is influenced by the nectar of the plant sources. The initial nectar-producing plant collects minerals from the earth, and these minerals eventually end up in the nectar (Felsner *et al.*, 2004) [13]. Ash content is an important standard for determining the botanical origin of honey. There have been claims that the variations in ash composition are caused by the floral origin (Johnson *et al.*, 1950) [14].

**Conclusion**
The results showed higher variability in the composition of honey from stingless bees of different species, predominantly flowering and geographical origin. The geochemical and geographical differences were probably related to the variations of the physico-chemical components of honey samples.

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