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A comparative quality evaluation of honey made by *A. dorsata and A. cerena indica* from the Melghat region of Maharashtra.

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Abstract:

The physicochemical properties of the honey samples were studied in this experiment. Squeezed honey from *A. dorsata* and *A. cerena indica* were collected from experimental beehives placed across in Melghat region. Physico-chemical aspects like electrical conductivity, specific gravity, PH, glucose-fructose ratio, moisture content, total protein content, free acidity (formic acid), ash content, HMF value, and minerals were tested and all common characteristics of honey were present. *A. dorsata* honey has a higher protein content and glucose-fructose ratio than *A. cerena indica* honey. The higher values of electrical conductivity of honey from *A. cerena* were found. The findings and physicochemical properties of honey samples are match with the values of Food Safety and Standard Authority of India (FSSAI). According to the data acquired by comparing the two samples of honey, several physicochemical traits of *A. dorsata* are more valuable than that of *A. cerena indica*. They may vary according to the comb's location, floral supplies, water accessibility, environmental conditions, the weather, and other considerations. They might also change over time.

Keywords: Honey**,** *Apis dorsata*, *Apis cerena indica*, Melghat,

Introduction

One of the most complex meals generated by nature is honey, which is also the only sweetener that can be consumed by people unprocessed (Iglesias, M.T. *et al.,* 2004). It is a naturally delicious material that bees make from plant nectar. Bees gather it, change it by mixing it with other substances, deposit it, hydrate it, and then leave it in honeycomb to ripen and develop (Council of European Union, 2002). Honey has beneficial nourishing, restorative, and preventative qualities (Pereira, P.C.M. *et al.,* 1998). These characteristics can be explained by the physical and chemical makeup of the object. Sugars make up the majority of honey's chemical composition, accounting for 82% of its total weight (Chang, H. G. *et al.,* 1988). The composition of honey is influenced by the kind of flowers that bees visit, the climatic conditions in which plants develop, and the plants' maturity (Abu-Tarboush, H. M. *et al.* 1993 & Anklam, E. 1998). Since the hive's foraging area is more than 7 km^2, the bees are exposed to air, soil, and water, and the concentration of minerals in honey reflects the quantity of those minerals in the entire area (Przybylowski & Wilczynska 2001, Atrouse, O. M. *et al.* 2004). The honey business values physicochemical examination of honey since these elements are closely linked to storage quality, granulation, texture, flavour, and the nutritional and therapeutic benefits of honey. The climatology, agronomy, and floristic characteristics of the Melghat region are completely distinct from those of the other districts.

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According to the "Flora of Melghat Tiger Reserve," there are 650 naturalised plant species in total, including 90 trees, 66 shrubs, 316 herbs, 56 climbers, 23 sedges, and 99 grasses. 200 of them were discovered to have therapeutic efficacy (Dhore & Joshi 1988). Numerous forest rangers, botanists, taxonomists, and scholars investigated the Melghat region often and made contributions to the region's flora. There are 117 families, 547 genera, and 1008 species listed in the most recent "Checklist of Flora of Melghat: 2018-19" (Khedkar & Atre 2019), more than 550 of which have been recorded for their ethnobotanical and medicinal benefits.
Apis dorsata is the largest of the honeybee species. also known as the rock bee or giant honeybee. They have the distinctive behaviour of building a single comb that can be quite large, often hanging from high tree branches or cliffs. Giant honeybees are known for their defensive behaviour when their nest is threatened. *Apis cerana indica* is a subspecies of the Eastern Honeybee (Apis cerana). They are smaller in size compared to *Apis dorsata* but larger than *Apis mellifera* (Western Honeybee). These bees build their nests in tree cavities or similar sheltered locations. Apis cerana indica is known for its adaptability to various climates and is commonly found in South Asia Abrol, D. P. (1997).

The physicochemical properties of honey encompass its characteristics such as water content (typically 17–20%), density, acidity (pH 3.2–4.5), viscosity, colour influenced by floral sources, and antioxidant-rich composition. Honey's natural acidity, low water content, and various compounds contribute to its microbial stability and resistance to spoilage. Additionally, honey's flavour, aroma, and electrical conductivity vary based on its botanical origin and sugar content, while crystallization is a natural process influenced by factors like temperature. Overall, these properties collectively define honey's unique composition and qualities Abrol, D. P. (1997).

To learn more about the physico-chemical characteristics of honey from the *Apis dorsata* and *Apis cerena indica* species in the Chikhaldara region, the present study was conducted.

Material and Methods

Sampling area:- Chikhaldara (Melghat) is a tribal area surrounded by dense forests in the Satpuda hills. It encompasses both the Gugamal National Park and the Sanctuary of Wan and Melghat, which are located in the two tehsils of the Amravati district, namely Chikhaldara and Dharni (Bhoyar *et al.*, 2018). One of the most significant forest products in Chikhaldara is honey (Melghat). The wild honey bees, or *Apis dorsata*, are the source of almost all the honey produced in the Melghat. The great variety of the Melghat woodlands creates a favourable environment for the development of honey bees (KVIC, 2019).

Honey sample:- Five samples of raw squeezed honey made by the *Apis dorsata* and *A. cerena indica* in different parts of Chikhaldara were obtained from skilled beekeepers. Until analysis, the samples were kept in sealed plastic containers at room temperature.

pH: The pH of a 10% (w/v) solution of honey produced in milli-Q water (Millipore Corporation, Billerica, Massachusetts, USA) was measured using a pH metre (HI 98127, Hanna Instruments, Mauritius).

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Honey colour analysis: Using the Pfund classifier, the colour intensity of honey samples was determined. In a nutshell, homogenous honey samples free of air bubbles were poured into a 10-mm light-path cuvette until it was almost halfway full. A colour photometer (HI 96785, Hanna Instruments, Cluj County, Romania) was used with the cuvette. In order to compare colour grades to a standard of analytical-grade glycerol, colour grades were given in millimetre (mm) Pfund grades. Utilising the United States Department of Agriculture's (USDA) authorised colour standards, measurements were made in triplicate for each sample (USDA 1885).

Specific gravity: Clean and completely dry the specific gravity bottle before weighing. Fill it to the appropriate level with freshly boiling, quickly cooled, and 27 °C-maintained pure water, then weigh it. Remove the water, thoroughly dry the bottle, and then fill it with a sample of honey that has been kept at the same temperature. Re-weigh the bottle.

Acidity in Formic acid: Take 10 g of the sample and dissolve it in 75 ml of carbon dioxide-free water in a suitable titration flask. Completely combine. Use 4 to 6 drops of thoroughly neutralised phenolphthalein solution to titrate against a standard sodium hydroxide solution (the pink hue of the indicator should last for at least 10 seconds).

Electrical conductivity: An HI 98311 conductivity metre (Hanna Instruments, Mauritius) and a 20% (w/v) solution of honey suspended in milli-Q water were used to measure electrical conductivity and total dissolved solids (Oddo, L. P. *et al.*, 2004). The milli-Q water's electrical conductivity was found to be less than 10 S/cm. Each sample was examined in triplicate for electrical conductivity and total dissolved solids; the mean values were given in mS/cm and ppm, respectively.

Moisture content: A refractometric approach was used to calculate the moisture content. In general, as a sample's solid composition rises, so does its refractive index. Using an Atago handheld refractometer (KRUSS, HRH30, Hamburg, Germany), the refractive indices of samples of honey were determined at room temperature. The data were then corrected for the reference temperature of 20°C by using a correction factor of 0.00023/°C. Wedmore's table was used to calculate the percentage of moisture content that corresponds to the corrected refractive index after the moisture content was measured three times by Helrich, K. (1990).

Ash content: To measure the amount of ash, a clean, empty crucible was heated to 6000 degrees Celsius for one hour in a muffle furnace, cooled in a desiccator, and then its weight was recorded (W1). In a crucible (W2), one gramme of each sample was taken. With the use of a blowpipe, the sample was ignited over a hob and burned until blackened. After that, the crucible was heated to 5500 C in a muffle furnace for 2-4 hours. All of the sample's organic material has completely oxidised, as evidenced by the presence of grey-white ash (BIS 1994 second addition).

Protein content: Using Lowry's technique (Lowery O. H.) the protein content of the honey was determined. Briefly, 1 mg/mL stock solutions were diluted to create BSA solutions with final concentrations ranging from 0.05 to 1.00 mg/ml. dilutions, 0.2 mL of protein solution was added to several test tubes, and 2 mL of the analytical reagent alkaline copper sulphate was then added. The resulting mixture was thoroughly mixed, and it was then incubated for 10 minutes at room temperature. After that, each tube received 0.2 mL of the reagent Folin-Ciocalteau solution, which was then incubated for 30 minutes. At 660 nm, the absorbance was measured after the colorimeter had been calibrated with a blank.

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Hydroxy methyl furfural (HMF): High-performance liquid chromatography (HPLC) is used to measure HMF. An HPLC technique based on the International Honey Commission (IHC) method was used to measure HMF concentrations (Shapla U. M. *et al.*, 2018). A 0.45 nm nylon membrane filter was used to filter the honey samples, each weighing 10 g, before they were injected (20 ml) into an HPLC system (Waters 2695, Milford, MA, USA) fitted with a Photodiode Array Detector (PDA) (Waters 2996). A guard cartridge filled with a comparable stationary phase was attached to the Merck Purospher Star RP-18e HPLC column (150 4.6 mm, 5 ml), which was manufactured in Germany. The isocratic mobile phase used in the HPLC procedure had a flow rate of 1.0 mL/min and was composed of 90% water and 10% methanol. All of the solvents were HPLC-grade. With particular monitoring at 285 nm, the detection wavelength ranged from 200 to 450 nm. After accounting for the dilution of honey samples, the HMF concentrations in the samples were determined by comparing the peak regions of the samples with HMF standard solutions (Sigma-Aldrich, USA). The concentration and area of the HMF peaks were shown to be linearly related (r2 = 0.9997) (results are given in mg/kg).

Mineral content: Utilising a flame photometer, determine the amounts of sodium (Na), potassium (K), calcium (Ca), and lithium (Li). Principle: The emission of radiant energy that occurs when an element's atoms return to their ground state following their excitation by the flame's high temperature is what the flame photometer detects. The amount of emission is correlated with the element's concentration in the solution. Procedure: Flame photometry was used to analyse the sample's Na and K content (Isaac, R. A., & Kerber, J. D. 1971). For the determination of Na, Ca, and K, the same wet-digested food sample solutions as those used in AAS were employed. For Na, Ca, and K, standard solutions of 20, 30, 40, and 50 milli equivalent/L or ppm were employed (Gul, S., & Safdar, M. 2009).

Sugar: The volumetric approach of sugar analysis was used, and after reducing the non-reducing sugars in the sample, the reducing sugars were found using an iodometric measurement (Shaffer & Hartman, 1921). In short, 10 ml of honey was combined with 80 ml, of deionized water, five ml, of 10% sodium tungstate, and five ml, of 0.66-N sulfuric acid are added next. After shaking, the mixture was filtered. The cuprous titration was used to make the determination after adding 25 ml of Fehling-Soxhlet solution to 25 ml of the filtrate. Next, in order to convert copper into terms of reducing sugar, the Qisumbing and Thomas tables (Quisumbing & Thomas, 1921) were reviewed.

Result and Discussions:

Table No. 1

Sr. No.	Parameter	*A. dorsata*	*A. cerena indica*	FSSAI Values
1.	Colour (pfund scale) mm	69.5 ± 14.29	54.5 ± 7.18	-
2.	Electrical conductivity mS/cm	0.71 ± 0.04	0.79 ± 0.05	0.8
3.	Specific gravity	1.38 ± 0.11	1.23 ± 0.03	1.37
4.	pH	5.6 ± 0.35	5.27 ± 0.15	-
5.	Protein %	0.87 ± 0.01	0.59 ± 0.03	Max 1
6.	Moisture %	18.15 ± 0.25	20.2 ± 1.12	20
7.	Acids (formic acid) %	0.30 ± 0.03	0.22 ± 0.03	0.20

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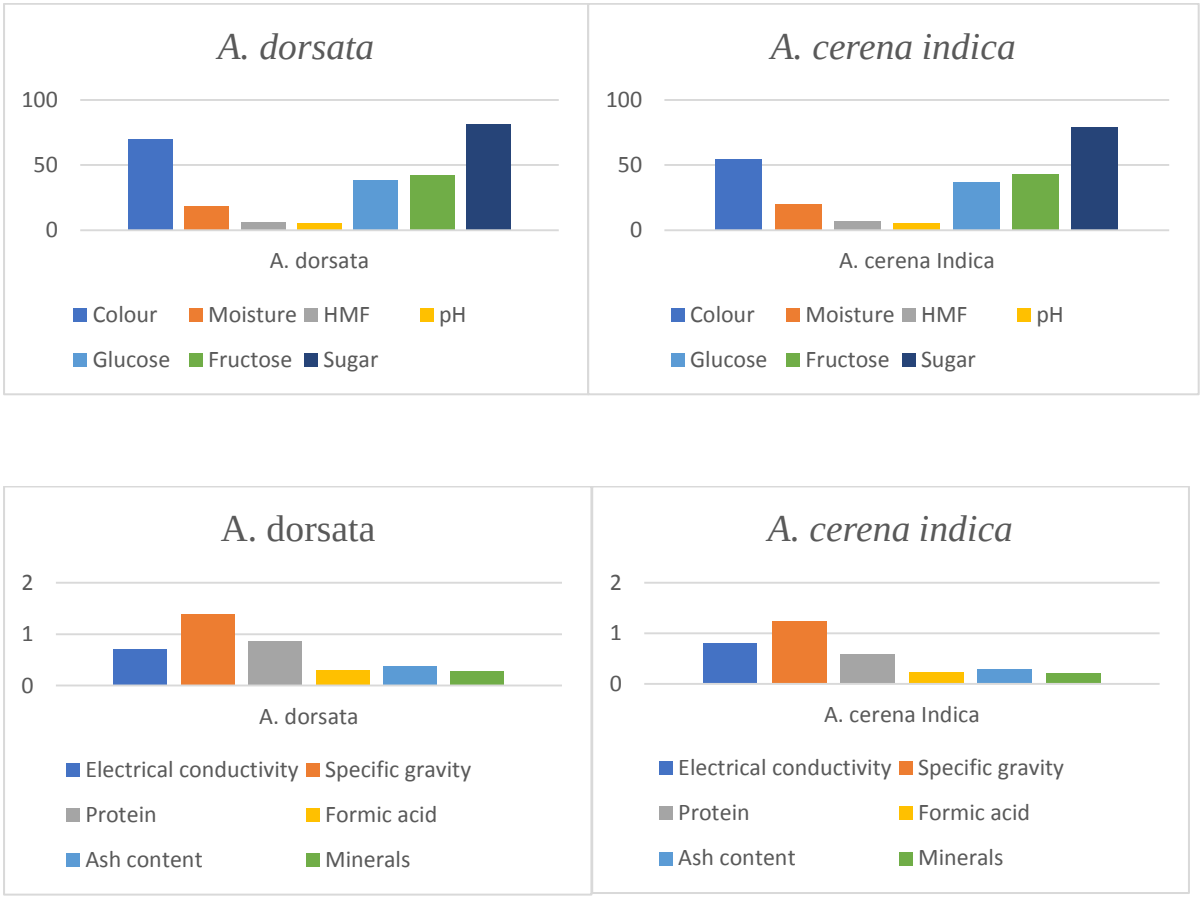
8.	Ash content %	0.38 ± 0.05	0.28 ± 0.05	0.5
9.	HMF mg/100 gm	6.12 ± 0.60	7 ± 0.27	8
10.	Glucose %	38.35 ± 1.10	36.82 ± 1.04	-
11.	Fructose %	42.31 ± 0.73	42.74 ± 0.80	-
12.	Sugar %	81.30 ± 0.28	79.24 ± 1.65	70
13.	Minerals %	0.28 ± 0.01	0.21 ± 0.04	-

Chart: 1



The colour of mustard and eucalyptus varied from 30 to 60 mm. Consequently, 21% of the Punjab sample's area had colour ranging from 30 to 40 mm, while 75% of the sample's area ranged between 40 and 55 mm (Kaur, P., *et al.*, 2016). From the Melghat region, honey that is collected has pfund values of 69.5 mm and 54.5 mm for *A. dorsata* and *A. cerena indica*, respectively. This range gives the light amber colour to the *A. dorsata* honey and the extra-light amber colour to the *A. cerena indica*.

Saxena & Sharma (2010) Study the physical and biochemical quality of some Indian honey. found the electrical conductivity at 0.66 mS/cm which is similar to 0.70 mS/cm, 0.79 mS/cm of *A. dorsata* and *A. cerena indica* respectively. And protein content 1980 ug/gm (0.19%/100 gm), which are similar to our values of 0.9%, 0.6% of *A. dorsata* and *A. cerena indica* respectively.

Krishnasree and Ukkuru (2017) have done the assessment of bee honey and estimate the specific gravity is 1.37 which is similar to Melghat honey that is 1.37, 1.22 of *A. dorsata* and *A. cerena indica* respectively. And free acidity is 0.20% which is similar to 0.27%, 0.23% *A. dorsata* and *A. cerena indica* respectively.

Mudasar, M., *et al.*, (2013) have done the chemical analysis of *A. cerena* from Jammu and Kashmir. They measured the pH with a digital pH metre and discovered that *A. cerena* has an acidic pH of 4.9, which is similar to Melghat honey of *A. cerena indica* pH of 5.3 and *A. dorsata* pH of 5.69. They also estimated the glucose and fructose contents to be 33.87% and 35.81%, respectively, and the ash content to be 0.87%, which is similar to Melghat honey of *A. Cerena indica* glucose fructose ratio 36.2 % and 38.5 %, ash content 0.37 % and *A. dorsata* glucose fructose ratio 31.6 % and 38.7% and ash content 0.27%.

Shah (2022) Analyzed the nutritional and chemical properties of Melghat honey, and estimated the HMF at 0.8 mg per 100 g by UV-VIS spectrophotometer, which is similar to 6.1 mg per 100 gm, 7 mg per 100 gm of *A. dorsata* and *A. cerena indica* respectively.

Yanniotis, S. (2006) They observed the effect of moisture content on the viscosity and found that 15–21% moisture is present, which is similar to 18%. And 20% of *A. dorsata* and *A. cerena indica* respectively.

Solayman M. *et al.*, (2016) determines the physicochemical properties, minerals, trace elements, and heavy metals in honey. They estimate the total mineral content of honey ranges from 0.11 to 0.72%, which is similar to 0.28 and 0.21% of *A. dorsata* and *A. cerena indica*, respectively.

CONCLUSION

The current study's objective was to assess and contrast the physico-chemical characteristics of a few honey samples from Maharashtra state's Meghat region. The outcomes demonstrated the high quality of each sample. This study is crucial for the marketing of regional honey and will help to understand the qualities of honey grown in the area. The composition of honey depends on the floral sources, which means nectar is present in the flower; the composition of the nectar also depends on the water, soil, temperature, and climatic conditions, so the composition of nectar varies at different locations. These all change the composition and colour of the nectar, and this ultimately changes the composition of the nectar, which is a sugar solution mostly composed of glucose, fructose and sucrose.

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