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**Research Article**

# ANALYSIS OF UNIFLORAL AND MULTIFLORAL HONEY FOR PHYSICO-CHEMICAL PROPERTIES IN SOUTHERN KARNATAKA, INDIA

**Razieh Almasi and Basavarajappa Sekarappa***

Apidology Laboratory, DOS in Zoology, University of Mysore, Mysore-570 008, India

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**ABSTRACT**

Honey is one of the main products of honeybee species, widely used as food throughout the world. India produces different types of honey which shows specific physico-chemical properties assuring acceptable quality as prescribed by the IHC. However, published reports on the physical properties and chemical constituents of unifloral and multifloral honey are poor and in the present investigation five physical properties and six chemical constituents were analyzed by following standard methods. Most of the physical properties viz., pH, specific gravity, electrical conductivity, absorbance, turbidity, and chemical constituents such as two monosaccharide's (fructose and glucose), proteins and ten minerals excepting moisture content and three vitamins revealed a significant difference between unifloral and multifloral honey in southern Karnataka. Results from the present investigation clearly indicated that forage collected by honeybees from flora available at diversified agro-climatic locations exhibit specific physical properties and chemical constituents in honey and which is unique to unifloral and multifloral honeys.



## INTRODUCTION

Honey is a sweet viscous amber coloured fluid produced by honeybee species. It is one of the internationally traded commodities (1), available with different brand names in India (2) and round the world. India is one of the mega biodiversity countries, possesses diversified ecosystems. Prevailed climatic conditions encouraged different honeybee species such as *Apis laboriosa*, *A. dorsata*, *A. florea*, *A. cerana, A. mellifera* and stingless bee's viz., *Trigona*, *Tetragonula* and *Melipona* species amidst temperate, tropical, sub-tropical ecosystems both in hilly and plain areas of India to produce unifloral and multifloral honey. Southern Karnataka is with diversified ecological conditions, created an immense potentiality for the production of unifloral and multifloral honey. Several researchers (3; 4; 5; 6) have reported the physical, biochemical, antioxidant and rheological properties in honey collected from *Apis* and *Trigona* species in India and other parts of the world. As it is essential to have internationally acceptable quality in the honey as prescribed by the international honey commission (1), regular analysis of honey for physico-chemical properties is needed.

India is one of the honey hubs, ranks eighth in honey production and first in beeswax production in the world (7). It produces more than 70 thousand tons of honey annually. Unifloral and multifloral honey from *Apis* species are being produced at different parts of Karnataka (8). Published reports on honey produced from domesticated honeybees such as *A. cerana* and *A. mellifera* in beekeeping activities at different agro-climatic locations of Karnataka are available (9). Similarly, reports on honey obtained from the natural colonies of *A. dorsata* are available (8). Besides, honey from specific plant species viz., *Eucalyptus, Syzigium*, *Litchi*, *Trifolium* and *Elettaria* etc, from specified agro-climatic locations (e.g. malnad, arid, semi-aird and plain areas) are also available (9). However, there are no published reports on the physical properties and chemical constituents of unifloral and multifloral honey. Interestingly, both unifloral and multifloral honey are exported to many foreign countries. Reports on the quality and contamination of these honeys are wanting in this part of the State. Recent past, Indian honey was rejected for export due to its contamination (10). Many apiculturists experienced setback due to honey rejection for international trade. Therefore, before qualify the honey for domestic use and export for international sale; it should be analyzed for physic-chemical properties. Since, honey is one of the international commodities; regular analysis has necessitated maintaining quality (11) and certification (2). Hence, present investigation was undertaken to screen the locally available different types of honey for their physical properties and chemical components.

**Corresponding author:* **Basavarajappa Sekarappa**
Apidology Laboratory, DOS in Zoology, University of Mysore, Mysore-570 008, India

## MATERIAL AND METHODS

***Physical properties of honey:*** The honey was collected from the shops at different agro-climatic locations where it is available as unifloral and multifloral honey in southern Karnataka. Collected honey was stored in airtight plastic containers until further analysis. The pH was measured by digital pH meter (CL54+, Toshcon Industries Pvt. Ltd. Hardwar) in a solution containing 5g of honey in 95ml of distilled water as per Nanda (3). The specific gravity = C-A/D-A, where, A = Weight of specific gravity bottle, C = Weight of specific gravity bottle with honey and D = Weight of specific gravity bottle with water was measured as per Nanda (3). A solution of 20g dry matter of honey was taken in 100ml distilled water and measured the electrical conductivity (EC) by using a digital electrical conductivity cell at $27^0$C and the results were expressed in millisiemens per centimeter as per Bogdanov (12). The absorbance and turbidity was determined in Elico Scannig Mini Spec, SL 177 Spectrophotometer as per Basavarajappa and Savanurmath (13).

***Chemical constituents in honey:*** The glucose content was estimated by using GOD/POD method as per Barham and Trinder (14) and Tenscher and Richterich (15). Similarly, the fructose content was estimated by using Resorcinol-HCl method as per Ashwell (16). The total protein content was determined by following the method of Lowery *et al.* (17). The minerals, moisture and vitamins were estimated by following standard methods. The collected data was analyzed and compiled by following standard methods (18).

## RESULTS

***Physical properties:*** The pH of unifloral honey was 4.16, whereas the multifloral honey pH was 4.66. The specific gravity was almost same (1.40 g/cm$^2$) in unifloral and multifloral honeys. The electrical conductivity (EC) revealed different values. It was high (0.867mS/cm) in multifloral honey and it was low (0.53mS/cm) in unifloral honey. Interestingly, the absorbance was high (1.52) in unifloral honey and it was low (1.42) in multifloral honey at 359nm (Table 1). However, the turbidity in multifloral honey was very high and it was almost doubled (6.01%) compared to unifloral honey, where it was 3.24%. Analysis of variance of physical properties revealed a significant difference (F=8.49; P>0.05) existed between unifloral and multifloral honey in southern Karnataka (Table 1).

***Chemical components:*** The fructose, glucose and mineral content differed much between the unifloral and multifloral honey, excepting total protein and moisture content (Table 2). The fructose content was 62.40 and 74.01 mg/ml respectively in unifloral and multifloral honeys. Similarly, the glucose content was 51.47 and 49.62 mg/ml in unifloral and multifloral honeys respectively. However, the moisture content was ranged in between 18.1 and 18.5%. The total protein content was 3.94mg/ml in unifloral honey and 3.1mg/ml in multifloral honey. However, the mineral content varied much and it was high (27.14 mg/ml) in unifloral honey and low (9.51 mg/ml) in multifloral honey (Table 2). Obviously, chemical constituents such as fructose, glucose, total protein, minerals and moisture content revealed a significant difference (F=50.02; P>0.05) between unifloral and multifloral honey in southern Karnataka (Table 2).

***Mineral content in honey:*** The Calcium (Ca), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Potassium (K), Sodium (Na) and Zinc (Zn) content differed considerably in unifloral and multifloral honeys (Table 3). Amongst minerals, K was very high (10.69 µg/ml) and it was followed by Ca (10.64 µg/ml), Na (3.35 µg/ml), Mn (1.38 µg/ml) and P (1.22 µg/ml) in unifloral honey. The Fe and Zn content was 0.66 and 0.16 µg/ml respectively in unifloral honey. Moreover, the Cr and Cu content was (0.10 µg/ml each) and Mn content was 0.08 µg/ml) in unifloral honey (Table 3). Accordingly, K, Ca, Na, Mg and P were referred as 'major minerals', Fe and Zn were referred as 'minor minerals' and Cr, Cu and Mn were referred as 'trace elements' in unifloral honey. Further, Na was very high (3.28 µg/ml) and it was followed by Ca (3.06 µg/ml) and K (1.30 µg/ml). However, the Mg, Fe and P content was 0.60, 0.12 and 0.11 µg/ml in multifloral honey. Moreover, the Cr, Cu, Mn and Zn content was 0.10 µg/ml each in multifloral honey (Table 3). Obviously, Na, Ca and K were referred as 'major minerals', Mg, Fe and P were referred as 'minor minerals' and Cr, Cu, Mn and Zn were called 'trace elements' in multifloral honey. Although, the pattern of occurrence of different minerals in unifloral and multifloral honey showed considerable variations, but didn't exhibit significant difference (F=1.295; P>0.05) between minerals (Table 3).

***Vitamins content in honey:*** Few vitamins namely: ascorbic acid, niacin and panthothenic acid were recorded below detection limits (BDL) i.e., o.o1µg/ml in unifloral and multifloral honey (Table 4).

**Table 1** ANOVA for physical properties of unifloral and multifloral honey

| Sl.No. | Physical properties | Unifloral Honey | Multifloral Honey | Mean ±SD | 'F'value |
|---|---|---|---|---|---|
| 1. | pH | 4.16 | 4.66 | 4.40 ± 1.65 | |
| 2. | Electrical Conductivity (mS/cm) | 0.53 | 0.80 | 0.66 ± 0.63 | |
| 3. | Specific gravity (g/cm$^2$) | 1.40 | 1.41 | 1.40 ± 0.93 | 8.49S (P>0.05) |
| 4. | Absorbance at 359 nm | 1.52 | 1.42 | 1.47 ± 0.94 | |
| 5. | Turbidity (%) | 3.24 | 6.01 | 4.62 ± 1.65 | |

Note: Each value is a mean of five observations.
S: Value is significant at 5% level.

**Table 2** ANOVA for chemical properties of unifloral and multifloral honey

| Sl.No. | Chemical properties | Unifloral Honey | Multifloral Honey | Mean ±SD | 'F'value |
|---|---|---|---|---|---|
| 1. | Fructose conent (mg/ml) | 62.40 | 74.01 | 68.20 ± 4.12 | |
| 2. | Glucose content (mg/ml) | 51.47 | 49.62 | 50.54 ± 3.55 | |
| 3. | Total Protein content(mg/g) | 3.94 | 3.10 | 3.52 ± 0.93 | 50.02S (P>0.05) |
| 4. | Minerals content (mg/ml) | 27.14 | 9.51 | 18.32 ± 2.14 | |
| 5. | Moisture (%) | 18.50 | 18.10 | 18.3 ± 2.13 | |

Note: Each value is a mean of five observations.
S: Value is significant at 5% level.

**Table 3** ANOVA for mineral components in unifloral and multifloral honey

| Sl.No. | Minerals (in µg) | Unifloral Honey | Multifloral Honey | Mean ±SD | 'F'value |
|---|---|---|---|---|---|
| 1. | Calcium (Ca) | 10.64 | 3.06 | 6.85 ± 1.30 | |
| 2. | Chromium (Cr) | 0.10 | 0.10 | 0.10 ± 0.15 | |
| 3. | Copper (Cu) | 0.10 | 0.10 | 0.10 ± 0.15 | |
| 4. | Iron (Fe) | 0.66 | 0.12 | 0.39 ± 0.31 | |
| 5. | Magnesium (Mg) | 1.38 | 0.60 | 0.99 ± 0.49 | |
| 6. | Manganese (Mn) | 0.08 | 0.10 | 0.09 ± 0.15 | 1.295NS (P>0.05) |
| 7. | Phosphorus (P) | 1.22 | 0.11 | 0.66 ± 0.70 | |
| 8. | Potassium (K) | 10.69 | 1.30 | 5.99 ± 1.22 | |
| 9. | Sodium (Na) | 3.35 | 3.28 | 3.31 ± 0.91 | |
| 10. | Zinc (Zn) | 0.16 | 0.10 | 0.13 ± 0.18 | |

Note: Each value is a mean of five observations.
NS: Value is significant at 5% level

**Table 4** Vitamins recorded in unifloral and multifloral honey

| Sl.No. | Vitamin | Honey type | |
|---|---|---|---|
| | | Unifloral | Multifloral |
| 1. | Ascorbic acid | BDL | BDL |
| 2. | Niacin | BDL | BDL |
| 3. | Panthothenic acid | BDL | BDL |

Note: Each value is a mean of five readings.
BDL: Below Detection Limit (0.01µg/g).

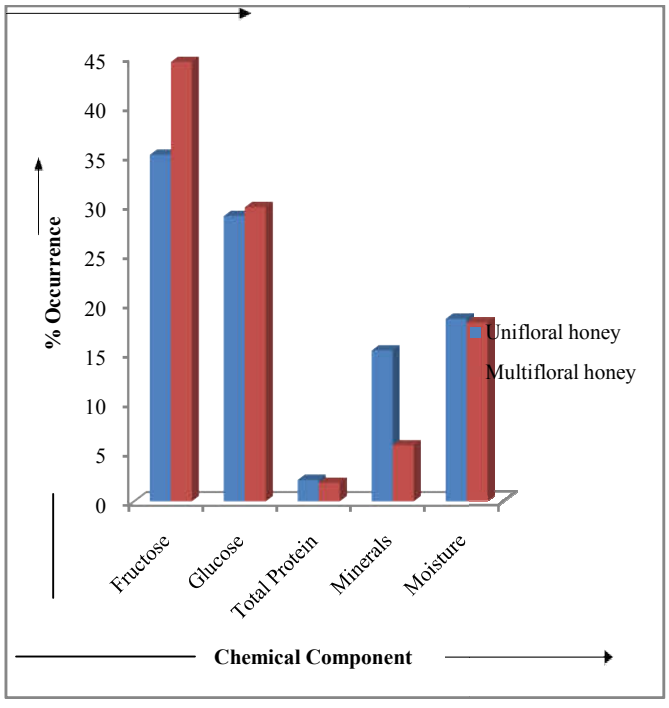


**Figure 1** Per cent occurrence of chemical constituents in unifloral and multifloral honey

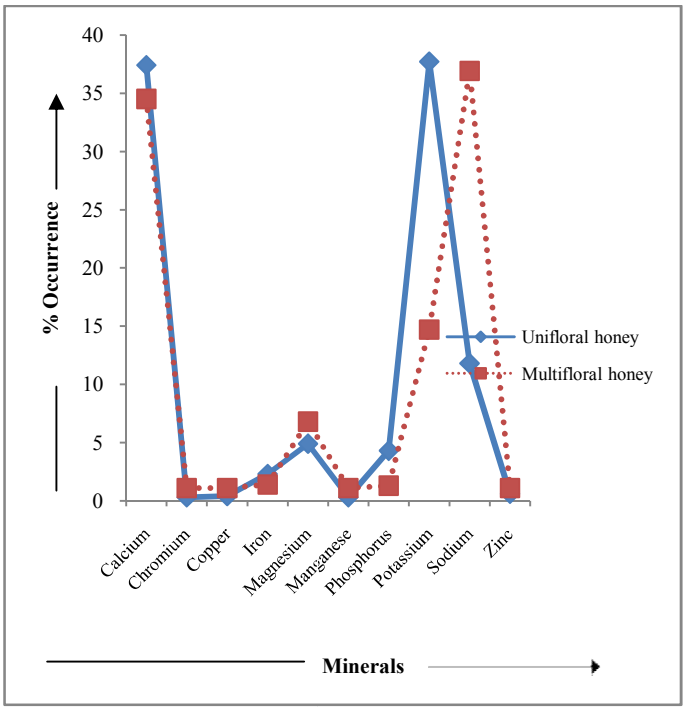


**Figure 2** Per cent occurrence of few minerals in unifloral and multifloral honey

## DISCUSSION

Honey is one of the important international commodities, used by people residing at different parts of the world. Therefore, it should be with good quality and free from any contamination or adulteration. To achieve quality standards as prescribed by the (1), various physical properties and chemical constituents are considered by various researchers (9). The pH analysis is essential to retain quality in honey (4). The pH value varies and usually it is in acidic condition. The acidic nature of honey depended on the sources of flora (19; 20) on which honeybees foraged. Honey collected from semi-arid, arid and malnad locations revealed acidic condition and showed in between 3.54 and pH 3.76 (21). During the present investigations, 4.16 to 4.66 pH was recorded and it is acidic. Despite the variation in geographical locations, in general, honey is acidic. Similar types of observations were reported by many authors. The specific gravity is used to determine honey quality (2). It varies much based on different agro-climatic locations. Several researchers have recorded 1.33 to 1.43g/cm$^2$ in unifloral honey (3; 19; 22; 23). Multifloral honey collected from semi-arid, arid and malnad locations revealed in between the range 1.32 and 1.37g/cm$^2$ in southern Karnataka (21). During the present investigation, the specific gravity was 1.40 to 1.41g/cm$^2$ recorded and it exhibited similar trend in both unifloral and multifloral honey. Hence, results are on par with the observations of the previous researchers. Unifloral and multifloral honey revealed variation in electrical conductivity (EC). The multifloral honey collected from different agro-climatic locations showed variations and it was in the range of 0.58 to 0.67mS/cm (21). However, the EC values varied considerably compared to earlier reports. The EC was high (0.80mS/cm) in multifloral honey and low (0.53mS/cm) in unifloral honey collected from different agro-climatic locations of southern Karnataka. The EC is most important parameter used to discriminate honey samples (24) and included recently as one of the parameters to decide the international standards

for honey (1). Hence, it becomes useful tool while classifying the honeys. Because, EC is indirectly reveals the botanical origin of honey and used routinely in honey analysis (25). The varied EC in unifloral and multifloral honey indicate the floral variation existed amidst different agro-climatic locations. Similar type of observations was made by Acquarone (26). Perhaps, this could be one of the reasons for the difference in EC of unifloral and multifloral honey in southern Karnataka. Further, multifloral honey collected from different agro-climatic locations showed absorbance in between 1.88 and 2.17 at 359 nm. During the present investigation, absorbance was within this range. It was high (1.52) in unifloral honey and little less (1.42) in multifloral honey. Absorbance measurement in honey indeed a specific parameter, help identify the colour and honey status (i.e., fluid state or crystal state) (27). As colour of honey is often an indication of its geographical origin (5), varies greatly based on the flora and the climate (28: 4). Perhaps, different agro-climatic locations might have influenced the floral source (29; 30) and in turn might have brought substantial variation of absorbance in unifloral and multifloral honey. Our observations are similar to the earlier reports published by Juszczak (5) and Muli (4)**.** Turbidity was drastically varied between unifloral and multifloral honey. It was almost doubled in multifloral honey (6.01%) compared to unifloral honey (3.54). Similar types of observations were reported in arid, semi-arid and malnad regions of southern Karnataka (9). Multifloral honey is dark in colour, contains excess amount of pollen grains and beeswax particles (31). Moreover, in certain parts of southern Karnataka, multifloral honey is harvested from *A. dorsata* colonies, where facilities for immediate transportation, proper preservation and maintaining quality in the harvested honey are poor (30). Perhaps it could have influenced the turbidity more in multifloral honey. However, in unifloral honey, it is produced from beekeepers and chances of pollen grains and beeswax particles contamination is less as it is critically attended by beekeeper under human inhabited conditions. Hence, turbidity is less in unifloral honey.

The fructose and glucose are commonly found monosaccharide in honey. More fructose content helps maintain fluidity in honey and its content should be more than glucose (32). Similarly, during the present investigation, more fructose was found in both unifloral and multifloral honey. However, quantity of fructose and glucose differ much in unifloral and multifloral honey in southern Karnataka. Honey is a mixture of fructose and glucose with minor amounts of organic acids, traces of minerals and vitamins (3). The average ratio of fructose to glucose is 1.2: 1 (28). Present findings almost near to the earlier published reports. Since, fructose and glucose are easily digestible and palatable food constituents, provides substantial energy (22). Hence, their analysis is very essential to meet the international standards as prescribed by the Alimentarius (1). Other sugars such as saccharose, maltose, trehalose and elizitose are of minor importance and hence they are not considered in the present study. The southern Karnataka experiences diversified agro-climatic conditions (29), which might have brought substantial variation in flower composition. Perhaps, it could have brought the change in the concentration of sugars like fructose and glucose in unifloral and multifloral honey. Analysis of protein content in honey is used to evaluate the quality (33) and help reveal the geographical origin of honey. The total protein content in unifloral and multifloral honey didn't show much variation in southern Karnataka. Usually, Indian honeys show less protein content (>5mg/g) (28). In general, protein content was >4mg/g with little variation in unifloral honey (3.94mg/g) and multifloral honey (3.10mg/g) in southern Karnataka. Khalil (19) and Chanchao (34) have reported low level of protein 0.8 to 2.2mg/g in various types of honeys namely: commercial brand honey, unifloral honey and multifloral honey. However, present study indicated the moderate level (3.10 to 3.94mg/g) of protein. Further, ten minerals were recorded first time in unifloral and multifloral honey in southern Karnataka. The minerals content varied considerably between unifloral and multifloral honey. This substantial variation perhaps influenced by the specific floral composition on which honeybees depended for their forage amidst diversified agro-climatic locations. Furthermore, unifloral and multifloral honeys also showed three vitamins namely ascorbic acid, niacin and pantothenic acid which were present below detection limit (0.01µg/g). Overall observations clearly demonstrated that, unifloral and multifloral honeys indicated considerable differences compared to previously published reports. Thus, these honeys are with specific chemical constituents which are unique to southern Karnataka.

## CONCLUSION

Results from the present investigation clearly indicated that forage collected by honeybees from flora available at diversified agro-climatic locations exhibit specific physical properties and chemical constituents in honey and which is unique to the honey type. Hence, unifloral and multifloral honey samples are although produced by honeybees, but characterized by specific physical properties and chemical constituents. It could help differentiate and identify honey type with specific apicultural value to sale in local and international market for consumers use.

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