Current Biotica 5(2): 144-156 ISSN 0973-4031

Physico-biochemical analysis of multifloral honey of *Apis dorsata* Fab. (Hymenoptera : Apidae) in southern Karnataka, India

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ABSTRACT

The multifloral honey of giant honeybee, *Apis dorsata* Fabricius (Hymenoptera: Apidae) was collected after the harvest from its colonies by honey hunters in the wild at Chamarajanagar, Kodagu and Mysore districts of southern Karnataka during 2009-2011. Physico-biochemical analysis of sixteen honey samples revealed that, *A. dorsata* honey contain a mixture of sugars namely glucose (51.68 ± 0.41g/100g), fructose (59.03 ± 0.65g/100g), that are in a ratio of 0.88 ± 0.02 with the total invert sugars 110.72 ± 0.82 g/100g. The specific gravity was 1.35 ± 0.02 g/cm^2 and the electrical conductivity was 0.63 ± 0.03. The multifloral honey expressed 0.24 ± 0.01% turbidity with an absorbance of 2.22 ± 0.38 at 359nm. The total protein content was 2.21 ± 0.79mg/g with a pH 3.68 ± 0.1. The results indicated that, *A. dorsata* honey has internationally acceptable quality with little variation in its physical, biochemical constituents. These variations may be addressed by developing targeted honey hunter education programs to improve honey harvesting and storage practices.

KEY WORDS: *Apis dorsata,* biochemical characters, multifloral honey, physical characteristics

INTRODUCTION

Honey is a natural sweet viscous fluid produced by honeybees from the nectar of flowering plants, which they collect and transform by combining with their salivary secretions, and deposit, dehydrate and store in the honey comb to ripen (Codex, 2009). Honey consists of different sugars, predominantly fructose and glucose and other substances such as organic acids, enzymes and solid particles derived from the nectar collected from different plants (Saxena *et al*., 2010)**.** Accordingly, there is a great variation in composition and characterisitics of multifloral honey due to difference in floral source (Anupama *et al*., 2003 and Joseph *et al*., 2007), geographical origin and climate (Anklam, 1998).

India has immense potentiality for the production of multifloral honey in the wild. The multifloral honey comes mainly from the colonies of giant honeybee, *Apis dorsata* Fab. In India, more than 90% of the honey is collected from *A. dorsata* colonies, harvested by tribes, forest dwellers and professional honey hunters in different ecosystems (Basavarajappa, 1998, 2004, 2007 and 2011; Basavarajappa *et al.,* 2009; Reddy and Reddy, 1989; Bradbear and Reddy, 1998; Setty and Bawa, 2002; Sattagi *et al*., 2002). Honey of *A. dorsata* is dark in colour, contains excess amount of pollen grains and beeswax particles (Rao *et al.,* 1998), available in most of the Indian markets (Tripathi, 1998). Moreover, it is known for its special components produced from the forest plants including variety of

medicinal plants (Rao, 1973; Basavarajappa and Raghunandan, 2010) and thus honey of *A. dorsata* has good nutritional and medicinal properties along with high religious value in rural India (Varadarajan, 2002).

Researchers from different parts of the world (Table 1) have found various types of honey that differ substantially in their physico-biochemical composition. It depends on the nectar source (Joseph *et al.*, 2007 and Muli *et al*., 2007), climate (Anklam, 1998) existed at different geographical regions. Honey is an internationally traded commodity (Anonymous, 2010), regularly analyzed for its physico-chemical properties, because these characters are important to determine quality and certification (Rehman *et al*., 2008). Several workers have studied different properties of Indian honey collected from various sources (Table 1). Saxena *et al*. (2010) have reported the physical, biochemical and antioxidant properties of some Indian honeys. Nanda *et al*. (2003) have studied the physico-chemical properties and estimated the mineral content of honey collected from different parts of northern India. Joshi *et al*. (1998) have made mellitopalyonological investigations on *Apis* and *Trigona* species honey collected in and around Pune. Rao *et al*. (1998) have emphasized the special needs while handling and processing of *A. dorsata* honey. Urska *et al*. (2009) have reported the sensory and pollen characteristics in multifloral honey collected from wild honeybees in Slovenia. However, the physico-chemical parameters of natural honey namely the moisture, saccharose and hydroxymethyl furfural (HMF) contents, acidity and electrical conductivity are strictly defined and which constitutes the quality indicators characterizing the different honey types (Juszczak *et al.,*2009). Obviously, the different honey types of the world show great variation in their physico-chemical and biological composition (Muli *et al*., 2007). Therefore, it is necessary to study the physico-chemical composition of honey (Urska *et al.,* 2009), which is greatly influenced by the geographical region, floral source and climate (Anklam, 1998; Joseph *et al*., 2007 and Muli *et al*., 2007).

Since, honey is one of the internationally traded vital commodities with an estimated global production 1.17 million metric tons (Anonymous, 2010 and Nanda *et al*., 2003), produced in most of the countries around the world (Mendes *et al.,* 1998). India, annually produces about 70,000 tones of honey, of which 25,000 to 27,000 tones is being exported to more than 42 countries including European Union, Middle East and the United States of America (Anonymous, 2007). However, in recent years Indian honey is rejected for export due to antibiotics contamination (Narian, 2010). Although India produces good quantity of honey that is consumed on a large scale basis, but there are no standards for antibiotics in honey (Anonymous, 2010). Moreover, there are only few reports available on the comparative physico-biochemical properties of Indian honeys (Saxena *et al*., 2010). Internationally acceptable quality in the honey would be maintained only by continuous evaluation for its constituents by conducting physico-chemical analysis (Joshi *et al*., 2000). The multifloral honey collected by cutting a piece of comb or complete comb directly from the colonies of *A. dorsata* is most popular among locals of different districts of southern Karnataka. It is most widely used in local consumption and sales. Therefore, the present investigation was carried out to anlayse the physico-bio chemical parameters of honey extracted from *A. dorsata* colonies.

MATERIALS AND METHODS

The multifloral honey was collected from *A. dorsata* colonies after harvest in the wild by honey hunters at Chamarajanagar, Kodagu and Mysore districts of southern Karnataka during 2009-2011. Five each honey samples from Chamarajanagar and Kodagu districts, six honey samples from Mysore district, altogether sixteen honey samples were collected and stored in airtight plastic containers until analysis as per Ouchemoukh *et al.* (2007). To study the physical and bio-chemical parameters, each sample was analyzed three times separately to get concordant value and following were the methods employed in the present study.

1. **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 **(**Nanda *et al*., 2003**).**

2. The **specific gravity** was determined by the method described by Nanda *et al.* (2003).

$$\text{Specific gravity of honey} = \frac{C-A}{D-A}$$

Where, A = Weight of specific gravity of bottle, C = Weight of specific gravity of bottle with honey and D = Weight of specific gravity bottle with water.

3. **Electrical Conductivity (EC):** A solution of 20g dry matter of honey was taken in 100ml distilled water and measured the EC by using a digital electrical conductivity cell at 27^0 C. The results were expressed in millisiemens per centimeter as per Bogdanov *et al*. (2004**).**

4. **Absorbance and turbidity** of honey samples was determined in Elico Scannig Mini Spec, SL 177 Spectrophotometer as per Basavarajappa and Savanurmath (2001).

5. **Estimation of Glucose content** in honey was done by using GOD/POD method as per Barham and Trinder (1972) and Tenscher and Richterich (1971).

6. **Estimation of fructose content** in honey was conducted by using Resorcinol-HCl method as described by Ashwell (1957).

7. **Invert sugar content** and ratio of glucose and fructose was estimated as per Guler *et al.* (2007).

8. **Total Protein content** of honey was determined by following the method of Lowry *et al.* (1951).

The collected data was analyzed and complied by following standard methods as described by Saha (1992).

RESULTS AND DISCUSSION

The pH:

The analysis of pH in the honey is considered as one of the quality factor used in the international honey trade (Muli *et al*., 2007). The pH values of multifloral honey of *A. dorsata* collected from different districts of southern Karnataka ranged from 3.54 to 3.76. The honey samples collected from Mysore district showed lower pH (3.54) and it was slightly higher in the honey

samples of Chamarajanagar (pH = 3.76) and Kodagu (pH = 3.74) districts (Table 2). Despite the variation in geographical origin, in general, multifloral honey of *A. dorsata* was acidic. As the acidic nature of honey depends on the sources of flora (Khalil *et al*., 2001; Thrasyvoulou and Mankis, 1995), that didn't much differed in southern Karnataka. Hence, the honey produced by *A. dorsata* at different districts of southern Karnataka is acidic in nature.

Specific gravity:

Specific gravity is one of the physical quality parameters, used to determine honey quality (Rehman *et al.*, 2008). The specific gravity was highest (1.37g/cm^2) in the multifloral honey of *A. dorsata* at Chamarajanagar district followed by Mysore (1.36 g/cm^2) and Kodagu (1.32 g/cm^2) districts respectively (Table 2). Thus, the specific gravity in multifloral honey was in the range of 1.32 and 1.37g/cm^2. To compare, other authors reported the following ranges for various types of honeys: 1.36 to 1.43g/cm^2 – Unifloral honey (Nanda *et al*., 2003), 1.33 to 1.36g/cm^2 - Unifloral honey (Khalil *et al*., 2001), 1.41 to 1.42g/cm^2 – Unifloral honey (Kamal *et al*., 2002), 1.43 ± 0.008 g/cm^2 – commercial honey (Nanda *et al*., 2003) and 1.40 to 1.41g/cm^2 – Unifloral honey (Ouchemoukh *et al*., 2007). Hence, values of the present investigations are in conformity with the results of the previous researchers. However, variation in specific gravity differs among various types of floral honey (Kamal *et al*., 2002), which is influenced by the time of extraction from the comb, ripening process and harvesting methods. In certain parts of southern Karnataka, multifloral honey is harvesting from *A. dorsata* colonies amidst forest ecosystem, where facilities for immediate transportation, proper preservation and maintaining quality in the harvested honey are poor (Basavarajappa, 2011). These problems should be addressed by beekeeping organizations to maintain quality in multifloral honey of *A. dorsata*.

***Electrical Conductivity (EC)*:**

Multifloral honey of *A. dorsata* revealed variation in electrical conductivity. The honey of *A. dorsata* in Mysore district has the highest EC (0.67mS/cm) compared to the honey of Kodagu district (0.58 mS/cm), whereas, the honey of Chamarajanagar showed 0.65mS/cm (Table 2). Thus, the EC of multifloral honey of *A. dorsata* at southern Karnataka was less than 0.70mS/cm. Since, EC is considered as most important parameter to discriminate honey samples (Guler *et al*., 2007) that included recently in the new international standards for honey (Codex, 2001). It becomes useful tool for classification of honeys. Moreover, EC is a good criterion of the botanical origin of honey and in the contemporary times it is determined in routine honey analysis instead of the mineral content (Sahinler and Gul, 2004). The varied electrical conductivity in the multifloral honey of *A. dorsata* is to be considered as an important marker of the geographical origin (Acquarone *et al*., 2007) in this part of Karnataka.

***Absorbance*:**

Multifloral honey of *A. dorsata* collected from Chamarajanagar district shows highest absorbance (2.63 at 359 nm) followed by the honey of Mysore district (2.17 at 359 nm). However, multifloral honey of *A. dorsata* at Kodagu district showed 1.88 absorbance at 359nm (Table 2). However, *A. dorsata* honey of southern Karnataka revealed 2.22 ± 0.38 at 359 nm. Absorbance measurement of honey indeed a specific parameter, uses for identifying the

colour of honey and in turn reveal the honey status (i.e., fluid state or crystal state) (Anonymous, 2001). It is obvious that, colour of natural honeys is often an indication of their origin (Juszczak *et al*., 2009) that vary greatly based on the flora, geographical variation and climate (Anklam, 1998; Joseph *et al*., 2007 and Muli *et al*., 2007). As Chamarajanagar, Kodagu and Mysore districts experiences different climate and floral source (Basavarajappa, 2007 and 2011), perhaps, this might have interfered in substantial variation of absorbance of honey of *A. dorsata*. Our observations are in conformity with the observations of Joseph *et al*. (2007), Juszczak *et al*. (2009) and Muli *et al*. (2007)**.**

***Turbidity*:**

Table 2 shows the turbidity of multifloral honey of *A. dorsata* collected from southern Karnataka. The honey of Mysore district had highest turbidity (0.27%) followed by the honey of Kodagu district (0.24%) and Chamarajanagar district (0.22%). Thus, there was a considerable variation in the turbidity of honey found in different districts of southern Karnataka. Multifloral honey is dark in colour, contains excess amount of pollen grains and beeswax particles (Rao *et al.,* 1998; Tripathi, 1998), it influence the turbidity of honey. As it is one of the indicators of honey colour, its analysis help identify the quality.

***Reducing Sugar content*:**

Major sugars present in the multifloral honey are fructose and glucose. However, glucose content was less (51.25 to 51.75g/100g with a mean of 51.68 ± 0.41g/100gm) compared to fructose (58.39 to 59.69g/100gm with a mean of 59.03 ± 0.65g/100gm). It is obvious that, fructose content should be higher than glucose in order to maintain fluidity in the honey (Al *et al*., 2009). Thus, fructose was approximately more than that of glucose (Williams *et al.,* 2009). Our observations agree with the observations of Al *et al*. (2009), Juszczak *et al.* (2009), Saxena *et al*. (2010) and Williams *et al*. (2009).

***Ratio of Glucose: Fructose (G: F)*:**

The glucose and fructose ratio was high (0.88) in the multifloral honey of *A. dorsata* collected at Chamarajanagar and Kodagu districts followed by Mysore (0.87) (Table 2). On an average the glucose and fructose ratio was 0.88 ± 0.02. Thus, glucose and fructose ratio didn't differ much in southern Karnataka. However, multifloral honey is a mixture of fructose and glucose that are present in 14 to 20% with minor amounts of organic acids, traces of minerals and vitamins (Nanda *et al*., 2003). The average ratio of fructose to glucose is 1.2: 1 (White, 1978 and Anklam, 1998). Moreover, fructose and glucose are easily digestible and are more palatable food constituents, supplies substantial energy (Kamal *et al*., 2002). Thus, fructose and glucose are the most abundant sugars found in the honey, but other sugars such as saccharose, maltose, trehalose and elizitose are of minor importance. Hence, only fructose and glucose are considered in the present analysis.

Further, the wide variation in fructose-glucose ratio (1.03-1.33) indicating the variety of floral sources from which the honey samples originated. These variations and proportion of glucose to fructose in a honey might have depended largely on the source of nectar available in Chamarajanagar, Kodagu and Mysore districts of southern Karnataka. As these districts experiences different climate

(Basavarajappa, 2007), accordingly floral source varied considerably (Basavarajappa *et al*., 2009; Basavarajappa and Raghunandan, 2010). Perhaps, this might have brought substantial variation in fructose and glucose contents in the honey of *A. dorsata*. Our observations are in conformity with the observations of Joseph *et al*. (2007) and Muli *et al*. (2007)**.**

***Invert Sugar content (IS)*:**

The IS content in the honey was high (111.75) in Mysore district and it was followed by Chamarajanagar (110.77) and Kodagu (109.64) districts. Like, glucose and fructose ratio, invert sugar content was also didn't varied much (Table 2).

***Total Protein content*:**

The total protein content in the honey of *A. dorsata* shows a considerable variation. It was highest (3.11mg/g) in Mysore districts than that of the Chamarajanagar (1.86mg/g) and Kodagu (1.65mg/g) districts (Table -2). Generally, in Indian honeys, the protein content is normally less than 5mg/g (Anklam, 1998). However, honey of *A. dorsata* at Chamarajanagar and Kodagu districts exhibited low levels of protein (Table 2), where the honey was collected mainly from the colonies found at natural ecosystems such as forests, hills and rocky areas (Basavarajappa, 2011). The available flora in Chamarajanagar and Kodagu districts showed considerable variation in terms of species composition compared to Mysore district (Basavarajappa *et al*., 2009). The Mysore possess good climate and enriched with varied cultivable flora including natural flowering plants (Basavarajappa, 2011 and Rao, 1973). Since, natural honeys exhibit low levels of protein (Juszczak *et al*., 2009), which is dependent on the type of flora and thus it is variable (Saxena *et al*., 2010). Hence, our observations fall within the range typical of various types honeys which show varied protein content. To compare, other authors reported the following ranges for various types of honeys: 0.8 to 2.2mg/g in commercial brand honey (Saxena *et al*., 2010), 0.69 to 0.74mg/g in Unifloral honey (Khalil *et al,* 2001) and 1.6mg/100g in *A. dorsata* (multifloral honey) (Chanchao, 2009). Analysis of protein content in the honey is a new tool for evaluation of its quality (Mohammed and Babiker, 2009), this help identify the authenticity of honey in a given geographical region.

Further, the other physico-biochemical components evaluated in Unifloral honey (Khalil *et al*., 2001; Ouchemoukh *et al*., 2007; Rehman *et al*., 2008; Sahinler *et al*., 2004 and Williams *et al*., 2009), commercial brands of Indian honeys (Anupama *et al*., 2003; Juszczak *et al*., 2009; Nanda *et al*., 2003 and Saxena *et al*., 2010) and the multifloral honey of *A. cerana*, *A. mellifera* and *A. dorsata* (Joshi *et al*., 2000) (Table 1) have indicated similar values with slight variations compared to multifloral honey of *A. dorsata* (excepting fructose and glucose) collected exclusively from southern Karnataka (Table 2). Thus, the physical and biochemical properties of multifloral honey of *A. dorsata* at different districts of southern Karnataka revealed little variations in their physico-biochemical constituents compared to earlier reports (Table 1). The details of values of all the parameters studied by earlier workers may be obtained directly from the authors as given in the table 1. These disparities may be due to various ecological factors and man-made faulty operations. As the honey hunters/or beekeepers are not following uniform honey harvesting methods (Basavarajappa, 1998), honey is harvested by various people such as professional

honey hunters, tribals, who don't know the improved and new honey harvesting techniques (Basavarajappa, 2004). Further, the honey processing and storing practices are greatly varied in this part of State (Basavarajappa, 2011). Perhaps, all these factors might have altered the physico-biochemical properties of multifloral honey of *A. dorsata* in southern Karnataka. Similar type of variations were recorded by different honey analysts who have found substantial difference in the physico-biochemical constituents of honey (Ahmed *et al*., 2007; Al *et al*., 2009 Anupama *et al*., 2003; Joshi *et al*., 2000; Juszczak *et al*., 2009; Kamal *et al*., 2002; Khalil *et al*., 2001; Malika *et al*., 2005; Nanda *et al*., 2003; Ouchemoukh *et al*., 2007; Rehman *et al*., 2008; Sahinler *et al*., 2004; Saxena *et al*., 2010 and Williams *et al*., 2009). Further, climate also affects the composition and properties of honey (Anklam, 1998; Joseph *et al*., 2007 and Muli *et al*., 2007). Thus, quality of honey is of great concern for every country. Therefore, proper monitoring in the quality of honey is the top most priority in the years to come. If honey harvesting techniques and honey storing methods are improved at the field itself while harvesting the honey from wild colonies, internationally acceptable quality can be restored in multifloral honey of *A. dorsata*. Moreover, these disparities should be addressed by concerned authorities by conducting training cum-educative programs to beekeepers or honey hunters especially who are collecting honey from *A. dorsata* colonies in the wild. This may help facilitate to prevent fraud, to protect authenticity in multifloral honey. Because, honey is one of the important international commodities, it is essential to maintain internationally acceptable quality and composition (Joshi *et al*., 2000). As honey is offered by almost everybody in every country in the world, it is very important energy food (Mendes *et al.,* 1998) for different age groups.

CONCLUSION

It could be concluded from the above results that multifloral honeys of *A. dorsata* in terms of physico-biochemical components are appropriate to the quality standards of international honey trade in southern Karnataka. Despite poor harvesting and storing practices, *A. dorsata* honey possesses moderate physico-biochemical components which are appropriate to the quality standards of TSE and CODEX. The minor differences among the multifloral honey samples can be explained that origin of flora, the faulty processes in harvest and storage of honey by hunters such as prolonged and unsuitable storage conditions. The better quality honey appropriate to CODEX and TSE standards would be produced by training the beekeepers, honey hunters and beekeeping organizations on the importance of physico-biochemical contents of multifloral honey for human health and export.

ACKNOWLEDGEMENTS

The first author acknowledges the financial support extended by University Grants Commission, New Delhi for conduct of the work. Authors are thankful to the Chairman, DOS, Zoology Department, University of Mysore, Mysore for facilitating the present investigation.

Table 1: Physico-biochemical parameters of honey studied by various researchers

Sl. No.	Country	Source of Honey	Parameters studied	Reference
1.	India (Karnataka)	Multifloral honey (*Apis dorsata*)	pH, Sp. gr., EC, OD, Turbidity, Fructose (F), Glucose (G), Ratio (F:G), Invert sugars (G+F) & Total protein content	Present work
2.	India	Commercial brands of honey	pH, EC, Sp. gr., Moisture & Proteins	Saxena *et al*., 2010
3.	Poland	Commercial samples of honey	pH, EC, Fructose, Glucose & Proteins	Juszczak *et al*., 2009
4.	Nigeria	Unifloral honey	pH, EC & Moisture	Williams *et al*., 2009
5.	Romania	Honey (Fluid & Crystallized)	EC, Fructose, Glucose & F:G Ratio.	Al *et al*., 2009
6.	Pakistan	Unifloral honey (*A. melllifera)*	pH, Sp. gr. & Moisture	Rehman *et al*., 2008
7.	India	Multifloral honey	pH & Moisture	Ahmed *et al*., 2007
8.	Algeria	Unifloral honey	pH, EC, Fructose (F), Glucose (G), F:G Ratio, Moisture & Proteins	Ouchemoukh *et al*., 2007
9.	Moracco	Uni & Multi floral honey	pH, EC & Moisture	Malika *et al*., 2005
10.	Turkey	Unifloral honey	pH &. EC	Sahinler *et al*., 2004
11.	India	Commercial honey	pH, Moisture & reducing sugars	Anupama *et al*., 2003
12.	India	Commercial samples of honey	Sp. gr. & Moisture	Nanda *et al*., 2003
13.	Pakistan	Unifloral honey	pH, Sp. gr. & Moisture	Kamal *et al*., 2002
14.	Bangladesh	Unifloral honey	pH, Sp. gr., Moisture & Proteins	Khalil *et al*., 2001
15.	Nepal	Multifloral honey (*Apis dorsata)*, Unifloral honey (*A. cerana* & *A. mellifera)*	pH, EC, Moisture, Fructose (F), Glucose (G) & F:G Ratio	Joshi *et al*., 2000

Table 2: Physico-biochemical analysis of *A. dorsata* honey collected from southern Karnataka

Sl. No.	Districts	pH	Specific gravity (g/cm^2)	Electrical Conductivity mS/cm	Absorbance (OD at 359 nm)	Turbidity (%)	Sugar content (g/100g)		Ratios of G : F	Invert sugars (G+F)	Total protein content (mg/g)
							Glucose (G)	Fructose (F)			
1.	**C. Nagar**	3.76	1.37	0.65	2.63	0.22	51.75	59.02	0.88	110.77	1.86
2.	**Kodagu**	3.74	1.32	0.58	1.88	0.24	51.25	58.39	0.88	109.64	1.65
3.	**Mysore**	3.54	1.36	0.67	2.17	0.27	52.10	59.69	0.87	111.75	3.11
Mean ± SD		3.68 ± 0.1	1.35 ± 0.02	0.63 ± 0.03	2.22 ± 0.38	0.24 ± 0.01	51.68 ± 0.41	59.03 ± 0.65	0.88 ± 0.02	110.72 ± 0.82	2.21 ± 0.79

Note: Each value is a mean of 48 observations.

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[MS received 10 May 2011;
MS accepted 18 August 2011]