

Comparison of Physicochemical Characteristics of Honey Produced by *Apis florea* And *Apis dorsata* honey bee species from Pakistan

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Running Title

Left: Qamer S., and Khan, A.A

Right: Biochemical analysis of *A.dorsata* and *A.florea* honey

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SUMMARY

This study was aimed to compare the physicochemical characteristics of honey produced by *Apis florea* and *Apis dorsata* honey bees in Pakistan. A total of 64 honey samples (32 of each species) were collected from different localities of Bhakkar, Kalar Kahar and Shorkot. The predominant vegetation in these areas were *Eucalyptus*, *mulberry*, *Acacia*, *Zizipus Jojoba*, *Neem*, *Sheesham*, *Citrus* and *Mango*. The physicochemical methods used for the characterization of honey are those as recommended by International Honey Commission. Most of the *A.dorsata* honey samples had moisture content beyond the maximum limit, whereas, *A.florea* honey samples had moisture content within the limit of International Standards. *A.florea* honey's pH was more than *A.dorsata* honey. The mean electrical conductivity (0.823mS/cm) and free acidity (11.56meq/kg) in *A.florea* honey samples was higher than average EC (0.741mS/cm) and FA (9.16meq/kg) of *A.dorsata* honey. Lactone content was recorded as (4.81 meq/kg) in *A. florea* honey, lower than (5.30 meq/kg) lactone content in

A.dorsata honey. More proline content (559.15mg/kg) in *A.dorsata* than *A.florea* honey (362.72mg/kg). Average HMF content (43.01mg/kg) in *A.florea* and 118.22mg/kg were found in *Apis dorsata* honey samples. The range (6.97-15DN) of diastase number of *A.dorsata* honey samples was lower than *A.florea* honey. The present investigations concluded that honey produced by *Apis florea* and *Apis dorsata* showed various quality parameters close to the International Honey Standards, except moisture content in *Apis dorsata* honey which were beyond the recommended limit (21%) and could be used as distinguishing parameter of honey produced by this honey bee species.

Key words: Physicochemical, *A.dorsata*, comparison, honey, analysis, *A.florea*

INTRODUCTION

Honey is a complex mixture and presents very great variations in composition and characteristics due to its geographical and botanical origin (Joseph *et al.*, 2007). The physical properties and chemical composition of honey have been investigated by many workers (Sporns *et al.*, 1992; Lichtenberg-Kraag *et al.*, 2002; Iglesias *et al.*, 2004; Qamer *et al.*, 2005). Generally it is evaluated by a physico-chemical analysis of its constituents by means of several important constituents as they influence the storage quality, granulation, texture, flavor and the nutritional and medicinal quality of the honey (Joshi *et al.*, 1999). The composition of honey depends mainly on the type of plant source utilized by the bees as well as regional and climatic conditions (Abu-Tarboush *et al.*, 1993; Ferreres *et al.*, 1994; Sanz *et al.*, 1994; Russo-Almeida, 1997). The international honey commission (IHC) has proposed certain constituents as quality criteria for honey. These include moisture content, electrical conductivity, reducing sugars, amount of fructose and glucose, sucrose content, individual sugars, mineral, free acidity, diastase activity, HMF content, invertase activity, proline content and specific rotation (Bogdanov *et al.*, 1999; Joshi *et al.*, 2000a).

There are four honeybee species in Pakistan. Among them is *Apis cerana*, *Apis florea*, *Apis dorsata* are indigenous species (Crane, 1999; Muzaffar, 1999a). The fourth species, *Apis mellifera*, was introduced in Pakistan in 1977 (Ahmad *et al.*, 1982). Both *Apis dorsata* (commonly known as “doomna”) and *Apis florea* (common name is “choti maki”) are wild in nature and make hives in open places. Both have suffered pesticide losses mostly in cotton and sugar-cane growing areas, being reduced to 40,000-50,000 colonies and about 12,000-15,000 colonies, respectively. *A. florea* honey is widely used in rural areas as a natural medicine for eye diseases, stomach pain, joint pains, and headache.

This research was designed to:

- 1) To find *Apis florea* and *Apis dorsata* colonies on different floral sources and collect honey.
- 2) To evaluate the physicochemical properties of *Apis florea* and *Apis dorsata* honey from Pakistan.
- 3) To increase the scientific knowledge about *Apis florea* and *Apis dorsata* honey collected from Pakistan.

MATERIALS AND METHODS

All physicochemical determinations were carried out according to the European Honey Commission and International honey commission methods (Bogdanov *et al.*, 1999) and compared with that of International Honey Standards. pH and electrical conductivity were determined in a 10g/75ml solution of honey in deionized water. Free, lactonic and total acidities were titrated in the same solution used for pH measurement (AOAC, 1975). Water content was determined by refractive index and correlation with Chataway Charts (Chataway, 1935). Proline content of honey was determined according to Cough (1969). Winkler’s method was used for the determination of HMF content (Winkler, 1955). The diastase activity of honey samples was determined according to the procedure of Schade *et al.* (1958). ANOVA (Steel and Torri, 1981) was performed for statistical analysis of data. *A.dorsata* samples were

collected from Bhakkar district where as *A.florae* samples were collected from Bhakkar, Kallar Kahar and Shorkot districts of Punjab province of Pakistan

RESULTS

Physicochemical analysis

Table 1 and 2 shows the physicochemical analysis of 64 Pakistani honey samples produced by *Apis dorsata* and *Apis florea* collected from Bhakkar, Kalar Kahar and ShorKot, respectively. Table 3 express comparative accounts on honey analysis from both species of, Pakistan.

Moisture content:

The moisture content recorded in *Apis florea* honey samples were found in the range of 15.80-20.6%. Out of 32 honey samples only one had moisture content close to the recommended limit i.e. 21%. However, eighteen samples had the moisture content in the range of 17-18.6%.

The moisture content recorded in *A.dorsata* honey samples were found in the range of 17.8-24.6%. In addition, twenty six honey samples of *A.dorsata* honey had moisture content beyond the recommended limit i.e. 21%. Only one honey samples of giant honey bee had water content below 20%.

pH:

The pH of Pakistani *A.florea* honey was found in the range of 3.81 to 5.90. Fifteen samples had pH range between 5.04-5.9.

The pH of Pakistani *A.dorsata* honey samples was found in the range of 3.57-5.90 with the average of 4.76. pH range of (5.1-5.9) was found in fourteen while eight samples had pH in the range of 5.3-5.9.

Electrical conductivity:

The average EC of *A.florea* honey samples was as 0.823mS/cm. out of 32 samples nineteen had electrical conductivity above 0.90mS/cm however, less than 1.0mS/cm. In addition, eight honey samples EC range falls between 0.45-0.47mS/cm.

About thirteen honey samples of giant bee had electrical conductivity values above 1.0mS/cm. Another, six samples (no. 2, 3, 4 and 7) had EC in the range of 0.55-0.75mS/cm.

Free acidity:

Free acidity was also determined in *A.florea* and *A.dorsata* honey samples. Seventeen samples had free acidity less than 10meq/kg. while, three had FA range between 19-19.5meq/kg.

The mean free acidity for *A.dorsata* honey samples was 9.16meq/kg and in the range of 3.50-19.0 meq/kg. About twenty one *A.dorsata* honey samples had free acidity less than 10meq/kg. Free acidity values of all *A.florea* and *A.dorsata* honey samples met the limits set by International Honey Commission.

Lactone content:

The average (4.81 meq/kg) lactone content of *Apis florea* honey samples were estimated in the range of 0.5-12.75meq/kg. Nineteen *Apis florea* honey samples had lactone content below 5meq/kg. Twelve samples showed lactone content above 5meq/kg but less than 10meq/kg. Lactone content values of all *A.florea* honey samples lies within the limit set by International Honey Commission.

The average (5.30 meq/kg) lactone content of *A.dorsata* honey samples were estimated in the range of 0.5-14meq/kg. Lactone content below 5meq/kg was recorded in fifteen honey samples. About thirteen *A.dorsata* honey had lactone content up to 10meq/kg.

Total acidity:

Total acidity calculated for *A.florea* honey samples was recorded at an average of 16.37meq/kg (9.50-24.5meq/kg). Total acidity higher than 10meq/kg but lower than 15meq/kg was recorded in about fourteen *A.florea* honey samples. Similarly, seven honey samples had total acidity in a close range of 19.5-21.5meq/kg.

Mean total acidity calculated for *A.dorsata* honey samples was 14.45meq/kg. About sixteen honey samples had total acidity more than 10meq/kg but lower than 15meq/kg and six sample showed total acidity less than 10meq/kg.

Proline content:

The average (362.72mg/kg) proline content of *A.florea* honey samples were found in the range of 169.60-534.0mg/kg. In about nine *A.florea* honey samples proline content were more than 500mg/kg, while, in eight *A.florea* honey samples had proline content in the range of 200-250mg/kg,

The average (559.15mg/kg) proline content of *A.dorsata* honey samples was in the range of 342.4-839.04mg/kg. Nine and seventeen honey samples exhibited proline content in the range of 600-850mg/kg and 500-600mg/kg, respectively.

HMF content:

In order to check the freshness of Pakistani *Apis florea* and *Apis dorsata* honey samples, HMF content were also measured.

Average HMF content of 43.01mg/kg (10.8-145.53mg/kg) was estimated in *A.florea* honey samples. About twelve *Apis florea* honey samples showed HMF content more than 20mg/kg but less than 30mg/kg. Four samples had HMF content more than the recommended limit i.e. ≤ 60 mg/kg.

HMF content was estimated at an average of 18.22mg/kg (1.92-146.30 mg/kg) in *A.dorsata* honey samples. Twelve samples had HMF content less than 10mg/kg. About eight honey samples had HMF content more than 20mg/kg but less than 30mg/kg.

Diastase number:

The average diastase number of *A.florea* honey samples was found to be 15.77DN (9.09-22DN). In twenty three samples diastase number was more than 10DN but less than 19DN.

The average diastase number of *A.dorsata* honey samples was found to be 11.0DN (6.97-28.1DN). Sixteen honey samples had diastase number in the range of 10-15DN. While nine honey sample had DN lower than 10DN but higher than 8DN. Only one honey sample had diastase number (6.97) lower than minimum International limit i.e. ≥ 8 .

DISCUSSION

Moisture content:

The average (18.77%) moisture content of *Apis florea* honey samples recorded were within the limits of International Honey Standards, except in one honey samples. The moisture content obtained in the present study showed resemblance with the values (17.4%) obtained by Latif *et al.*, (1956) in Pakistani *Apis florea* honey samples and by values (16.5%) obtained by Phadke (1968) in Indian *Apis florea* honey samples. The moisture content of *Apis florea* honey samples were recorded in the range of 15.80-20.6%. The sample no. 12 showed the highest moisture content (20.6%) which is close to the recommended limit i.e. 21%. The higher (23.8%) moisture content in *Apis florea* honey has also been recorded previously by Nair *et al.*, (1950).

The average (21.7%) moisture content of *Apis dorsata* honey samples was recorded in the range of 17.8-24.6%. About twenty six samples had water content more than the limit prescribed by The International Honey Standards i.e. 21%. The higher moisture content in *Apis dorsata* honey has also been recorded previously by Qamer *et al.*, (2008) from four (23.5%, 23.99%, 23.91% and 22.2%) different forests of Nepal, 21% by Joshi *et al.*, (2000a), 20.9% (18.9-24.2%) by Phadke (1968); 21% by Malakar (1997), 23.1% by Laude *et al.*, (1991) and 27.8% by Minh *et al* (1971) from Philippines. These high water content in *Apis dorsata* honey could be because of the possible dilution of honey by rain water as this honey bee species forms comb high in open air (Joshi *et al.*, 2000a). Therefore uniformly high moisture content seems to be

the peculiarity of *Apis dorsata* honey and could be used to evaluate the honey on the basis of honey bee species (Qamer *et al.*, 2008).

Moisture content is highly important for the shelf-life of the honey during storage (Perez-Arquillue *et al.*, 1994) because a high moisture content causes honey to ferment and spoil. The amount of water in honey is a function of many factors involved in ripening, including weather conditions and the original moisture of the nectar, harvest season, the conditions of storage and the degree of maturity (Terrab *et al.*, 2003).

pH:

pH is a useful index of possible microbial growth. Most bacteria grow in a neutral and mildly alkaline environment, while yeasts and moulds are capable of growth in acidic medium (4.0-4.5) and do not grow well in alkaline media (Conti *et al.*, 1998). The mean values of pH samples agree with that reported in many countries by many investigators. The obtained (3.81-5.90) values of pH of *Apis florea* honey samples in present study showed close resemblance to the pH value (3.62-5.46) recorded by Anupama *et al.*, (2003) in the commercial honey samples of India and to the pH (4.20) reported by Malika *et al.*, (2005) in Moroccan honey samples. The obtained pH values, however, higher as compared to the ones obtained (3.10-3.77) by Atanassova *et al.*, (2009) in Bulgarian honey, (3.4-4.5) by Chefrour *et al.*, (2009) in Algerian honey, (3.50-4.21) by Conti *et al.*, (2007) in Italian honey and (3.29-4.05) by Khan *et al.*, (2006) in honey samples of different geographical origin including Pakistan. The acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin. The pH of honey is low enough to prevent the growth of many species of bacteria (Leveen *et al.*, 1973).

The average (4.76) pH of *Apis dorsata* honey samples was measured in the range of (3.57-5.99). The present values were within the range of 3.42-6.1 prescribed by White *et al.*, 1962. Honey samples with a pH above 5, can be considered to be of low purity and low quality (Rehman *et al.*, 2007). These values show remarkable resemblance to (3.4-6.1) those reported by Belitz and Grosch (1988). Qamer *et al.*, (2008) reported pH in *Apis dorsata* honey from four Terai forests, Nepal as 4.68 (4.3-5.1), 4.58 (4.3-4.7), 4.39 (3.7-4.6) and 3.8 (3.7-4.3). These pH values were, however, higher as compared to the values calculated by Joshi *et al.*, (2000 a) in the honey samples of *Apis dorsata* harvested from two different nesting sites (trees) in Chitwan district Nepal which were 3.68 and 4.06, respectively.

Electrical conductivity:

The average (0.823mS/cm) electrical conductivity of *Apis florea* honey samples obtained from this study was slightly higher than least limits of Codex Alimentarius (2001) and of Directive 2001/110/EC from Council of European Union for blossom honey (≤ 0.8 mS/cm). Atanassova *et al.*, (2009) calculated (117-428 mS/cm) EC values for Bulgarian honey. EC value of 0.05 mS/cm was reported by Rehman *et al.*, (2007) for Pakistani honey samples. An average (34.09 μ S/cm) EC value was reported by Omafuvbe and Akanbi, (2009) for Nigerian honey.

The electrical conductivity values reported by Qamer *et al.*, (2008) were 0.060 (0.44-0.70), 0.61 (0.51-0.74), 0.48 (0.19-0.54) and 0.22mS/cm (0.18-0.42mS/cm) for *Apis dorsata* honeys from four forests of Nepal. EC value of 0.558mS/cm was reported by Iglesias *et al.*, (2004). These EC values reported by these authors were lower than the EC of *Apis dorsata* honey samples found in present study. Joshi *et al.*, (2000a) recorded EC values of *Apis dorsata* honey as 0.96mS/cm, higher than the present study values. Electrical conductivity measures all ionisable organic and inorganic substances present in honey. It

has been reported to be related to the botanical origin of honey and very often used in routine honey control instead of the ash content (Malika *et al.*, 2005).

Free acidity:

The average (11.56meq/kg) free acidity value recorded in the present study was higher than (5.53meq/kg), reported by Bera *et al.*, (2009). The free acidity values were, however, lower than those obtained by Chefrour *et al.*, (2009) in Algerian honey and Khan *et al.*, (2006) in honey samples of different geographical origin including Pakistan. Latif *et al.*, (1956) from Pakistan, Nair *et al.*, (1950) from India and Phadke (1968) from India reported (0.18f, 0.22f and 0.27f respectively) formic acid values in *Apis florea* honeys.

Free acidity was also determined in *Apis dorsata* honey samples at an average of 9.16meq/kg (3.50-19.0 meq/kg). Qamer *et al.*, (2008) reported free acidity values 44.459meq/kg (26.5-51.59meq/kg), 43.169meq/kg (35.00-47.519meq/kg), 43.149meq/kg (33.5-60.009meq/kg) and 48.9meq/kg (39.5-61.00meq/kg) of Nepal's *Apis dorsata* honey. However, the free acidity values recorded from present study were found lower to the values of *Apis dorsata* honey recorded by Qamer *et al.*, (2008). Joshi *et al.*, (2000a) did not report free or total acidity in *Apis dorsata* honey samples collected from Chitwan district, Central Nepal.

Latif *et al.*, (1956) from Pakistan, Mitra and Methew (1968) from India and Phadke (1968) from India reported (0.16f, 0.19f and 0.25f respectively) formic acid values in *Apis dorsata* honeys. The acidity of honey contributes to its stability against microorganisms and to flavour (Malika *et al.*, 2005). The pH of the honey is not directly related to free acidity because of the buffering action of the various acids and minerals present (Abu-Tarboush *et al.*, 1993).

Lactone content:

Malika *et al.*, (2005) reported lactone content as 7.5meq/kg in Moroccan honey from different botanical origins including unifloral (*Eucalyptus*, *Thymus*,

Euphorbia) and multifloral types. Chefrour *et al.*, (2009) noticed the lactone value (3-39.8meq/kg) in Algerian honey. These lactone values were closer to the (2.7-14.1meq/kg) values reported by Daniela *et al.*, (2008) of Christ's thorn (*Paliurus spina christi* Mill) honey. The average (4.81meq/kg) lactone content of *Apis florea* honey samples in present study were less than those reported by above authors.

Lactone content range of *Apis dorsata* honey samples were lower to the values 13.2meq/kg (0.00-20.5meq/kg), 18.79meq/kg (11.5-18.00meq/kg), 15.14meq/kg (12.5-18.5meq/kg) and 3.5meq/kg (7.00-21.00meq/kg) reported by Qamer *et al.*, (2008). Iglesias *et al.*, (2004) noticed the lactone values in Spanish honey at an average of 5.08meq/kg, closer to the present lactone content of *Apis dorsata* honey samples.

Total acidity:

Latif *et al.*, (1956) from Pakistan (0.18*f*), Nair *et al.*, (1950) from India (0.22*f*), and Phadke (1968) from India (0.27*f*) studied total acids in *Apis florea* honey. Chefrour *et al.*, (2009) noted 10.25-87.46meq/kg total acidity in Algerian honey. Total acidity (37.20mq/kg) in Nigerian honey was calculated by Omafuvbe and Akanbi (2009). Total acidity of *Apis florea* honey in present study was lower than those reported by above authors.

Qamer *et al.*, (2008) reported total acidity values of 61.74meq/kg (30.00-71.50meq/kg), 67.98meq/kg (46.5-64.00meq/kg), 60.02meq/kg (46.00-77.00meq/kg) and 56.39meq/kg (53.00-79.50meq/kg) in *Apis dorsata* honey samples collected from four Terai forests, Nepal. The total acidity range obtained from present study were lower to the reported values of total acidity. Latif *et al.*, (1956) from Pakistan (0.16*f*), Nair *et al.*, (1950) from India (0.39*f*), Mitra and Mathew (1968) from India (0.19*f*) and Phadke (1968) from India (0.25*f*) reported total acids in *Apis dorsata* honey. Minh *et al.*, (1971) from Philippines reported total acidity of 40.2meq/kg as an average in *Apis dorsata* honey. The variation in acidity among different honey types may attributed to

either variation in the contents of organic and inorganic acids due to harvest season or floral types (El-Sherbiny and Risk, 1979).

Proline content:

Meda *et al.*, (2005) determined proline content varied from 437.82 to 2169mg/kg. Sorkun *et al.*, (2001) reported proline content as 68.85-116.10mg/100g in Turkish honey. Thrasyvoulou and Manikis (1995) investigated physicochemical characteristics of Greek unifloral honey of different botanical origins and reported proline contents as 324-673, 290-580, 430-734, 596-12.0, 305-650, 298-1199, 264-636, 329-931 (mg/kg), respectively. In *Apis florea* honey samples, proline content was estimated at an average of 362.72mg/kg (169.60-577.78mg/kg). Except in two *Apis florea* honey sample, proline content were according to the criterion of Codex Alimentarius (2001) and of Directive, 2001/110/EC, from council of European Union.

Qamer *et al.*, (2008) reported 98.38mg/kg (23.38-153.6mg/kg), 100.8mg/kg (20.65-330.0mg/kg), 119.98mg/kg (42.15-193.18mg/kg) and 160mg/kg (101.97-198.81mg/kg) of proline content of *Apis dorsata* honey samples from Nepal. The average (559.15mg/kg) proline content of *Apis dorsata* honey samples in present study was found higher than those reported by Qamer *et al.*, (2008). Joshi *et al.*, (2000b) reported 875.82ppm of proline content in *Apis dorsata* honey samples from Nepal. Sanchez *et al.*, (2001b) reported 76.0mg/100g and 81.1mg/100g proline in honey samples from two different geographical locations of Spain. Some authors have reported that high values for proline are typical for honeydew honeys (Diez *et al.*, 2004). The proline content has been found to vary considerably between different honeys (Bogdanov *et al.*, 1999).

HMF content:

HMF is one of the important quality criteria to determine the freshness and overheating of honey. In Pakistani *Apis florea* honey, average (43.01mg/kg) HMF content was close to the average (39.0mg/kg) HMF content reported by Bera *et al.*, (2008). The present study showed that 4 out of 32 samples of *Apis florea* honey exceeded the maximum limit described for HMF content by Codex Alimentarius (2001). Chefrour *et al.*, (2009) reported HMF content (1.728-480mg/kg) in Algerian honey. Williams *et al.*, (2009) investigated (34.26-36.42mg/kg) HMF for Nigerian honey. Meda *et al.*, (2005) measured HMF content (2.30-38.20mg/kg) of Burkina Fasan honey.

HMF content reported by Qamer *et al.*, (2008) were 33.36mg/kg (7.68-61.28mg/kg), 30.36mg/kg (3.84-57.60mg/kg), 36.48mg/kg (7.68-59.52mg/kg) and 56.10mg/kg (21.12-76.6mg/kg) from four Terai forests, Nepal. HMF content of *Apis dorsata* honey samples in current research were investigated at an average of 18.22mg/kg (1.92-46.30 mg/kg), lower than those reported by Qamer *et al.*, (2008).

Diastase number:

Diastase is a starch hydrolyzing enzyme in the honey. The diastase activities 15.77 (9.09-22) DN in *Apis florea* honey samples were well within the DN range recommended as quality criteria by and by Codex Alimentarius (2001) and Directive 2001/110/EC from Council of European Union. Azzedine *et al.*, (2009) reported 2.84-114 schade units of diastase in Algerian honey. Williams *et al.*, (2009) estimated (11.62-14.63DN) diastase numbers in Nigerian honey. Daniela *et al.*, (2008) analysed Christ's thorn honey and reported diastase number (25.39-50.51DN). Meda *et al.*, (2005) investigated diastase activities (6.5-62.3DN) in Burkina Fasan honey.

The present study indicates that except one sample, diastase activities in remaining *Apis dorsata* honey samples were well within the DN range recommended as quality criteria by Codex Alimentarius (2001) and Directive 2001/110/EC from Council of European Union. Diastase numbers reported by

Qamer *et al.*, (2008) were 27.69 (16.66-43) DN, 29.35 (18-42.85) DN, 25.49 (18.75-30) DN in the honeys of same species. Diastase numbers in present study were lower than those reported by above author.

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Table 1: Physicochemical analysis of honey produced by *A.dorsata* collected from Bhakkar, Punjab

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
pH	3.65	3.89	3.8	4.12	5.19	5.84	4.1	4.19	3.73	5.76	3.64	5.9	5.8	5.61	5.7	5.5	5.4	5.34	3.57	3.8
Moisture Content	20.6	21.4	17.8	21.4	23.8	23.4	18.2	21.8	21	22.2	21.8	24.6	21.4	20.6	21.8	22.6	23	22.2	23.4	21
EC (mS/cm)	0.296	0.315	0.307	0.312	1.143	1.113	0.308	1.098	0.292	1.085	0.285	1.098	1.094	1.102	1.036	1.08	1.094	1.086	1.26	1.56
FA (meq/kg)	15.5	14	18.5	13.75	3.75	3.5	6.25	10.75	8	5.5	7.75	6	5.75	4.75	5.75	6	6.25	6.5	19	16
LA(meq/kg)	7	4	5.5	5.75	0.5	3.75	4.25	2.5	0.5	9	4.25	2.5	1	4.5	5.5	6.5	5.5	6.5	13	14
TA(meq/kg)	22.5	18	24	19.5	4.25	7.25	10.5	11.25	8.5	14.5	12.25	8.5	6.75	10.5	11.75	13	11.75	13	32	30
Proline (mg/kg)	477.76	544.7	532.8	839	794.9	342.4	696.3	736.6	570	421.1	382.7	519.68	602.2	503	401.9	614.4	515.8	567	562.56	558.08
HMF(mg/kg)	21.5	23.42	25.53	4.99	6.45	27.07	28.03	7.29	8.83	6.72	14.3	7.68	2.88	11.52	16.51	2.49	1.92	4.8	2.49	8.06
Diastase No.	9.67	12	10.71	11.11	11.53	10	8.57	11.11	14.28	14.28	10.71	15	27.14	16.97	22.57	12.5	15	13.57	28.1	14.28

continued

Table 1: Physicochemical analysis of honey produced by *A.dorsata* collected from Bhakkar, Punjab

22	23	24	25	26	27	28	29	30	31	32	Mean	Codex draft 1999	EU draft 1999	Directive 2001/EC
5.6	5.6	5.61	5.7	4	4.8	3.8	3.5	4.8	4.64	4.1	4.76			
22	21.4	20.6	22.8	21.6	21	21	22.5	23	21.8	23	21.7	≤ 21%	≤ 21%	≤ 21%
0.765	0.668	0.52	0.55	0.67	0.34	0.48	0.71	0.72	0.485	0.8	0.74	≤0.8mS/cm	≤0.8mS/cm	≤0.8mS/cm
8.5	5.75	4.75	9.75	6	6.25	7.5	17.9	16	7.75	10.26	9.16	≤ 50meq/kg	≤ 50meq/kg	≤ 50meq/kg
2.5	1	4.5	5.5	6.5	5.5	6.5	7.1	11	3.25	2.5	5.30			
11	6.75	10.5	15.25	13	11.7 5	14	25	27	11	12.76	14.45			
559.6 8	612.2 4	503.0 4	504	614.4	592	567	676	558. 1	582.8	532	559.15	≥ 180mg/kg		
17.68	22.88	11.52	26.51	12.49	19.9 2	34.8	12.49	18.0 6	46.3	23.08	18.22	≤ 60mg/kg		
8.5	10.5	10	8.57	10.7	9	9	8.5	6.97	8	8	11.2	≥ 8		

*The suggested values for proline content and electrical conductivity for new honey standards (Bogdanov *et al.*, 1999)

** Council of the European Union. Council Directive 2001/110/EC of Dec. 20, 2001, relating to honey. *Off. J.Eur. Commun.*, 2002. Jan 12, L10/47-L10/52, FA (Free acidity), LA (Lactone), TA (total acidity), EC (electrical conductivity)

Table 2: Physicochemical analysis of *A.florea* honey collected from Bhakkar, Shorkot and Kalar Kahar, Punjab

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
pH	4.11	3.81	3.85	3.99	3.83	5.9	5.18	5.41	5.46	5.85	5.04	4.75	4.78	5.28	4.73	5.46	5.27	5.32	3.88	4.78
Moisture Content	17	17	18.6	16.4	17.4	15.8	19.4	18.2	18.2	19.4	18.6	20.6	17.8	19.4	19	19.4	16.2	19	18.2	19.8
EC(mS/cm)	0.685	0.455	0.452	0.45	0.455	0.956	0.962	0.897	0.946	0.936	0.964	1.003	0.998	0.98	0.959	0.93	1.023	0.961	0.474	0.977
FA (meq/kg)	12	19.5	19	18.5	17.5	10	9	7.5	9.5	9	11	9.5	10	8	10.5	8	9	7.5	17.75	8.5
LA (meq/kg)	9	2	0.5	2.5	3	3.75	2.5	2	3	12.75	9	7.5	1.5	4	0.5	3.75	4.25	9.5	6.25	9
TA (meq/kg)	21	21.5	19.5	21	20.5	13.75	11.5	9.5	12.5	12.75	20	17	11.5	12	11	11.75	13.25	17	24	17.5
Proline(mg/kg)	401.9	506.2	524.2	508.8	577.3	327.7	515.2	169.6	216.96	294.4	225.9	208	188.8	238.1	348.2	203.9	428.7	373.6	497.1	500
HMF(mg/kg)	145.5	24.19	23.23	22.65	25.34	25.92	24	23.23	26.88	25.34	23.61	25.15	13.95	32.83	48.16	36.09	52.99	29.95	10.8	30.84
Diastase No	12.5	13.63	15.78	14.28	12.5	13.04	11.53	10	9.09	17.64	21.42	15	12	14.28	20	21.42	20	18.75	17.64	13.04

(Continued)

Table 2: Physicochemical analysis of *A.florea* honey collected from Bhakkar, Shorkot and Kalar Kahar, Punjab

21	22	23	24	25	26	27	28	29	30	31	32	Mean	Codex	EU 1999	Drective
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												draft 1999	2001/EU		
4.18	3.8	3.19	5.25	5.1	5.32	5.28	5.48	5.77	4.27	4.81	4.85	4.81			
18.4	18.6	17.4	17.4	19.4	17.8	17.4	18	18.4	16.2	18.6	19.4	18.77	≤ 21%	≤ 21%	≤ 21%
0.962	0.452	0.45	0.936	0.936	0.998	0.98	0.927	0.93	0.933	0.452	0.921	0.823	≤0.8mS/c m	≤0.8mS/c m	≤0.8mS/cm
9	19	18.5	9	9	10	8	10.5	9.5	9.3	17	10	11.56	≤50meq/k g	≤50meq/k	≤ 50meq/kg
2.5	0.5	2.5	2.75	3.75	3.5	5.5	8	6.75	7.5	7.5	7.5	4.81			
11.5	19.5	21	11.75	12.75	13.5	13.5	18.5	16.25	16.8	24.5	17.5	16.37			
169.6	217	294.4	225.9	406	536	238.1	348.16	511	428.65	534	440	362.72	≥ 80mg/kg		
32	30.5	32	35.5	49	49	55.4	123	131.5	42.99	62	67	43.01	≤ 60mg/kg		
11.53	15.78	14.28	17.64	17.64	19	20	20	22	12	17	15	15.77	≥ 8		

*The suggested values for proline content and electrical conductivity for new honey standards (Bogdanov *et al.*, 1999)

** Council of the European Union. Council Directive 2001/110/EC of Dec. 20, 2001, relating to honey. *Off. J.Eur. Commun.*, 2002. Jan 12,L10/47-L10/52, FA (Free acidity), LA (Lactone), TA(total acidity), EC (electrical conductivity)

Table No. 3: Comparison of physicochemical characteristics of *Apis florea* and *Apis dorsata* honey from Punjab, Pakistan

Sr. No.	Parameter	<i>Apis florea</i>		<i>Apis dorsata</i>		Codex draft 1999	EU draft 1999	Directive 2001/EC **
		Average	Range	Average	Range			
1	pH	4.81±0.698	3.81-5.91	4.76±0.939	3.57-5.90			
2	Moisture content%	18.77±1.526	15.80-20.20%	21.7±1.666	17.8-24.6	<21%	<21%	<21%
3	EC (mS/cm)	0.823±0.227	0.450-1.023	0.741±0.400	0.285-1.56	≤0.8* mS/cm		
4	Free acidity (meq/kg)	11.56±4.246	7.5-19.5	9.16±5.041	3.50-19.0	≤50 meq/kg	≤40 meq/kg	≤50 meq/kg
5	Lactone content (meq/kg)	4.81±3.490	0.5-12.75	5.30±3.569	0.5-14			43
6	Total acidity (meq/kg)	16.37±4.438	9.50-24.5	14.48±7.591	4.02-32.0			
7	Proline content (mg/kg)	362.72±137.367	169.60-577.7	559.15±130.856	342.4-839.04	≥180* mg/kg		
8	HMF content (mg/kg)	43.01±32.104	10.8-145.53	18.22±31.424	1.92-46.30	<60mg/kg		
9	Diastase number	15.77±3.731	9.09-22.0	11.20±2.596	6.97-28.1	≥8		

“ANOVA”: Probability values at 5% for different physicochemical parameters for *Apis florea* and *Apis dorsata* honey. For moisture content P<0.01, P>0.05 for pH, P>0.05 for EC, P>0.05 for free acidity, P>0.05 for lactone content, P>0.05 for total acidity, P<0.01 for proline content, P<0.05 for HMF and P<0.01 for diastase number.