



PHYSICO-CHEMICAL CHARACTERISTICS AND CORRELATION ANALYSIS OF HONEY OF WILD HONEY BEES

MEENA THAKUR*, NEHA GUPTA, BHARTI THAKUR, HARISH KUMAR SHARMA AND KIRAN RANA

Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173 230, Himachal Pradesh, India

*Email: mnthakur15@yahoo.com (corresponding author): <https://orcid.org/0000-0001-9057-5745>

ABSTRACT

Honey of wild honey bees *Apis dorsata* and *Apis florea* was sampled from northern states of India during the year 2018-20 and analyzed for physico-chemical parameters and microbial load using standard procedures and correlation analysis. *A. dorsata* honey had high moisture content (17.20%), pH (6.25) and F: G ratio (1.98), whereas *A. florea* honey exhibited higher bacterial loads (3.82) than *A. dorsata* (3.70). No fungal spores were recorded in any of the honeys analyzed. Significant positive correlation was recorded between colour and pollen density; amino acid content and acidity; Vitamin C and acidity in honey of *A. dorsata*. Whereas Electrical Conductivity (EC) and pH; amino acid and acidity content in honey of *A. florea* had significant positive correlation. Bacterial load had significant positive correlation with pH and moisture. The quality parameters were well within the acceptable limit defined by the Food Safety Standards Authority of India.

Key words: Honey parameters, *Apis dorsata*, *Apis florea*, physico-chemical parameters, microbial load, wild honey, honey quality, honey bacterial loads, honey standards. honey composition

The hive product honey is considered as a symbol of prosperity and sanctity. It is considered a complete food containing a range of nutritionally important elements viz., saccharides, organic acids, amino acids, minerals, vitamins, aromatic substances, colour etc. (Sulbha et al., 2012). It is used as a popular sweetener in many commercially manufactured products, in cosmetics and medicines (Crane, 1980). In India, Bureau of Indian Standards (BIS) and Food Safety and Standards Authority of India (FSSAI) have specified the standards for quality parameters of honey. The major quality criteria for honey are the physical and chemical components of honey viz., moisture content, sucrose content, reducing sugars, pH value, EC, ash content, free acidity, diastase activity and HMF content, which are dependent on the geographical region, honey bee species, flower types, weather conditions, processing conditions, packaging and storage period (Tornuk et al., 2013; Escuredo et al., 2014) and accordingly they are correlated to each other. Moisture content of honey is a limiting factor in determining its quality and stability, acids in honey are related to colour and flavour of honey and its chemical properties such as acidity, pH and EC. Higher the acid content, higher will be the electrical conductivity. The hydroxymethylfurfural (HMF) and diastase content of honey are used as an indicator of honey adulteration. Honey harvested from hives which are fed with sucrose will have low diastase content.

Fructose in presence of acids produces HMF. If honey is fresh, HMF is low. It is an indication of heat exposure to honey and adulteration with artificial sugar syrup. Since, the honey flow sources vary with the altitude, the present study was therefore aimed to evaluate the physicochemical characteristics and bacterial load of honey of wild honey bees *Apis dorsata* and *Apis cerana* and to analyze the correlation between various quality parameters.

MATERIALS AND METHODS

Honey of wild honey bees (500 g) was collected from different locations of North India viz., Himachal Pradesh, Jammu and Kashmir, Haryana, Punjab, Rajasthan and Uttarakhand (Fig. 1) and analyzed for quality parameters using standard procedures viz., colour in spectronic-20; pH measured directly (AOAC, 2012), moisture content by oven drying method (Ranganna, 2007), sucrose content and fructose to glucose ratio by I.S.I. (1974) method, acidity by titrimetric method (AOAC, 2012), total phenolic content by Folin-Ciocalteu procedure (Bray and Thorpe, 1954), total H₂O₂ content by standard addition method (Pasiyas et al., 2017), HMF factor by Fiehe's test (ISI, 1974), acidity by titrimetric method (AOAC, 1984) and proline, (amino acid) by standard AOAC (1980). Microbial load was determined by Standard Plate Count

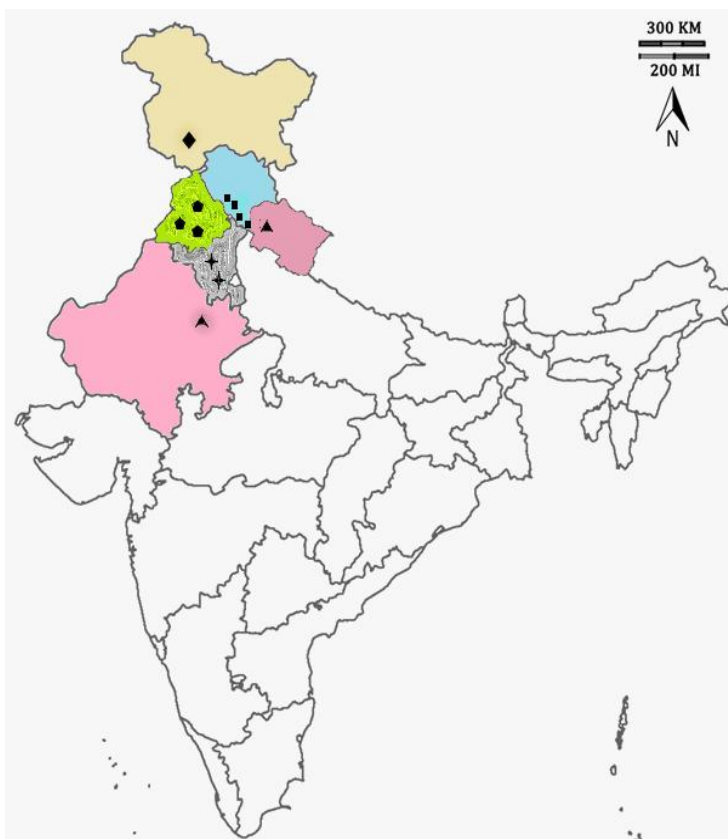


Fig. 1. Location map showing different sampling locations of honey

Technique (Wollum, 1982). The values recorded for various parameters were compared with standards for honey prescribed by different organization/authorities (White, 1975; FSSAI, 2018).

RESULTS AND DISCUSSION

Physico-chemical parameters and bacterial load of honey

The physical parameters viz., pollen density (pollen grains per 10 g), colour (OD at 560 nm), pH, EC (mS/cm) and moisture (%), respectively were 79500, 0.72, 6.25, 0.35 for *Apis dorsata* and 52250, 0.80, 4.66, 0.54 and 16.20 for *A. florea* (Table 1) The chemical parameters viz., sucrose (%), fructose (%), glucose (%), F:G, HMF, acidity, amino acid, Vitamin C, enzyme and phenol content, respectively were 5.65, 39.59, 20.01, 1.98, 40.00, 49.63, 74.74, 21.05, 20.24, 76.37 for *Apis dorsata* and 7.35, 31.86, 32.71, 0.97, 63.0, 83.56, 76.05, 24.51, 86.37 for *A. florea*. Whereas the bacterial load was 3.70 and 3.82 log CFU/g of honey, respectively for *A. dorsata* and *A. florea* honey. High moisture content, pH and F:G ratio was recorded for *A. dorsata* i.e., 17.20 (%), 6.25 and 1.98, respectively. The results are in

agreement with the finding of Balasubramanyam (2011) and Iftikhar et al. (2011). The acidity and pH of 83.56 meq/ kg and 4.66, respectively for *A. florea* honey is in accordance with the findings of Sulbha et al. (2012). Higher acidity is an indicator of sugar fermentation which is converted into organic acids (Gomes et al., 2011). Akram et al. (2014) and Qamer et al. (2008) reported diastase number in the range of 18.33-29.00 DN and 5.10- 29.00 DN, respectively which support the present data of 20.24 DN for *A. dorsata* honey.

In the present study, the EC value for *A. dorsata* honey was low (0.35 mS/ cm). Wild honey normally comes from honey hunters, while squeezing, adds impurities which might affect the EC and other properties of honey. The reason for variations in physico-chemical characteristics could be due to differences in honey flow sources and altitude. *A. florea* exhibited comparatively higher bacterial loads (3.82 log CFU/g of honey) than *A. dorsata* (3.70 log CFU/g of honey) (Table 1). The type of sample (retailed or raw), age of honey (fresh or old), season and type of analytical techniques used for harvesting of honey could be the probable reasons for this discrepancy in the bacterial

Table 1. Physico-chemical characteristics and bacterial load of honey of wild honey bees

Parameters	Honey bee species	
	<i>Apis dorsata</i>	<i>Apis florea</i>
Pollen density (Pollen grains per 10 g)	79500 (4.90)	52250 (4.72)
Colour (OD at 560 nm)	0.72	0.80
pH	6.25	4.66
EC (mS/ cm)	0.35	0.54
Moisture (%)	17.20	16.20
Sucrose (%)	5.65	7.35
Fructose (%)	39.59	31.86
Glucose (%)	20.01	32.71
F:G	1.98	0.97
HMF (mg/ kg)	40.00	63.00
Acidity (milliequi/ kg)	49.63	83.56
Amino Acid (mg/ 100g)	74.74	76.05
Vit.C (mg/ 100g)	21.05	24.51
Enzyme (Diastase/ DN)	20.24	22.24
Phenols (mg/ 100g)	76.37	86.37
Bacterial load(log CFU/ g of honey)	3.71	3.82

Table 2. Correlation coefficient of physical parameters of *Apis dorsata* honey

	Pollen density	Colour	pH	EC	Moisture
Pollen density	1.00				
Colour	0.06*	1.00			
pH	0.19	-0.59	1.00		
EC	-0.72	-0.60	0.05	1.00	
Moisture	0.88	-0.40	0.13	-0.33	1.00

(* Significant at 5%)

loads associated with the honey (Snowdon and Cliver, 1996). The bacterial load detected in the present study is comparatively lower than documented (5.5-24.0 log CFU/ g) by Adadi and Obeng (2017), however, lies in the range (1.47-4.6 log CFU/ g) documented by Hosny et al. (2009), Babarinde et al. (2011) and Kamal et al. (2019). Bacterial population density reported in the current study may be attributed to both the environmental as well as apicultural (harvesting and processing) factors (Kamal et al., 2019). No fungal isolates were found in any of the processed samples which supports the previous documentation (Omafuvbe and Akanbi, 2009).

Correlation analysis of physico-chemical parameters

The data in Table 2 indicates significant positive correlation between colour and pollen density of *A. dorsata* honey indicating that the colour of the honey

changes according to the floral source, mineral content and storage conditions as supported from the previous findings (Kaya et al., 2005). Significant positive correlation was observed between amino acid and acidity and between vitamin C and acidity content (Table 3). Honey contains ascorbic acid because most flowers on which the bees forage contain vitamin C which serves as an antioxidant. Therefore, antioxidant activity of honey, which depends on its botanical origin, is related to vitamin C content (Kesio et al., 2009). Significant positive correlation between EC and pH was observed for *A. florea* honey (Table 4). The electrical conductivity of honey depends on the content of inorganic salts, organic acids, proteins, complex sugars and mineral contents in the sample and thus may affect pH (Lullah-Deh et al., 2018). Significant positive correlation was recorded between amino acid and acidity of *A. florea* honey (Table 5).

Table 3. Correlation coefficient of chemical parameters of *Apis dorsata* honey

	Sucrose	Fructose	Glucose	F:G	Acidity	Amino acid	Vit.C	Diastase	Phenols
Sucrose	1.00								
Fructose	0.26	1.00							
Glucose	0.83	-0.50	1.00						
F:G	0.29	0.61	0.33	1.00					
Acidity	-0.82	0.11	-0.36	-0.17	1.00				
Amino acid	-0.92	0.02	-0.56	-0.27	0.98*	1.00			
Vit.C	0.60	-0.16	-0.07	-0.05	0.95*	0.85	1.00		
Diastase	-0.35	-0.75	-0.79	0.02	-0.24	-0.03	-0.47	1.00	
Phenols	-0.86	0.57	-0.99	0.22	-0.42	-0.60	0.15	0.78	1.00

(*Significant at 5%)

Table 4. Correlation coefficient of physical parameters of *Apis florea* honey

	Pollen density	Colour	pH	EC	Moisture
Pollen density	1.00				
Colour	0.89	1.00			
pH	0.87	-0.66	1.00		
EC	-0.88	-0.94	0.54*	1.00	
Moisture	0.39	-0.08	0.79	-0.09	1.00

(* Significant at 5%)

Table 5. Correlation coefficient of chemical parameters of *Apis florea* honey

	Sucrose	Fructose	Glucose	F:G	Acidity	Amino acid	Vit.C	Diastase	Phenols
Sucrose	1.00								
Fructose	0.16	1.00							
Glucose	0.63	-0.15	1.00						
F:G	0.57	0.29	0.32	1.00					
Acidity	-0.99	0.15	-0.51	-0.58	1.00				
Amino acid	-0.95	0.13	-0.35	-0.57	0.98*	1.00			
Vit. C	0.06	-0.05	-0.73	-0.09	0.21	0.38	1.00		
Diastase	-0.013	-0.06	-0.78	0.05	-0.13	-0.31	-0.99	1.00	
Phenols	-0.99	0.16	-0.62	0.58	-0.99	-0.95	0.08	0.10	1.00

(*Significant at 5%)

Correlation analysis of physico-chemical parameters with bacterial load of honey

Bacterial load was positively correlated with pH and moisture content of honey (Table 6) which was at par with studies conducted by Mahmoudi et al. (2012) and Adadi and Obeng (2017). However, a non-significant negative correlation was recorded for bacterial load with acidity, F:G ratio, sucrose, colour and phenol content of honey.

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Table 6. Correlation between physico-chemical characteristics and bacterial load of honey

Characteristics	Bacterial load (log CFU/ g of honey)	
	<i>Apis dorsata</i>	<i>Apis florea</i>
Moisture	0.93*	0.84*
pH	0.89*	0.98*
Acidity	-0.25	-0.30
F: G ratio	-0.20	-0.35
Sucrose	-0.16	-0.22
Colour	-0.24	-0.34
Phenols	-0.41	-0.46

(*Significant at 5%)

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