



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(6): 46-54

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Received: 15-09-2020

Accepted: 21-10-2020

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Quality analysis of *Apis cerana* and *Apis mellifera* honey from Himachal Pradesh, India

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Abstract

The honey of hive bees *Apis cerana* and *Apis mellifera* collected from different locations of Himachal Pradesh, India was analyzed for quality characteristics viz., pH, colour, moisture, sucrose, fructose to glucose ratio, acidity, phenols and hydrogen peroxide content. The analyzed honey was of good quality as it met the required standards of Indian and International markets. Comparative analysis confirmed that *A. cerana* honey had highest moisture (17.38%), OD (0.50), sucrose (3.33) and H₂O₂ (201.92 mg/kg) content as compared to *A. mellifera* honey. However, significantly highest acidity (36.32 meq/kg) and phenol content (82.40 mg/100g) was recorded for *A. mellifera* honey. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) was perceived as an efficient tool for classifying and discriminated the tested honeys based on their analyzed physico-chemical parameters.

Keywords: Honey quality, hive bees, *Apis cerana*, *Apis mellifera*, hive products

Introduction

The hive product honey is a natural sweet substance produced by honeybees from collected plant nectars, plant secretions and excretions of plant sucking insects, which are transformed, dehydrated and stored in honeycombs for ripening (FSSAI, 2017) [1]. It contains energy providing nutrients such as amino acids, vitamins, minerals, enzymes, organic acids, phenols, water and is a high-energy carbohydrate food (80 - 85 %) with easily digestible honey sugars (White & Doner, 1980) [2]. These constituents influence its storage quality, granulation, texture, flavour, nutritional and medicinal quality and are of great importance to the honey industry. The quality of honey is evaluated on the basis of standard quality parameters standardized by different authorities viz., FSSAI Standards for Honey, Codex Alimentarius, European Union and International Honey Commission etc. Further, the chemical composition of honey depends on the geographical location as, even for the same plant species the accumulation of phytochemicals is influenced by climatic conditions (sunlight and moisture) and soil characteristics.

In India, the total honey production per annum increased from 3.7 thousand metric tonnes in 2017 to 4 thousand metric tonnes in the year 2018 (Anonymous, 2019) [3]. United States, Saudi Arabia, Morocco and Bangladesh were the major importer of Indian honey. According to National Horticulture Board (2018) [4], the total honey production in Himachal Pradesh was approximately 5500 MT in the year 2017-18.

Himachal Pradesh is a mountainous state situated between 30° 22'40" to 33° 12'40" N latitudes and 75° 45'55" to 79° 04'20" E longitudes, covering an area of 55,673 km² in the North-Western Himalaya. It is a mountainous state with different agro-climatic conditions (temperature ranging from -5.0 to 40°C), vast repository of flora with profuse variation at intra and inter-species levels and represents one of the most important beekeeping areas in India.

The hive bees, *Apis cerana* and *Apis mellifera* are domesticated in the state mainly for honey and pollination services. Besides, various hive products. *A. cerana* is traditionally managed by farmers in Himalayan region from 300m - 3400m amsl with an average honey production of 3-5 kg per colony per year. *A. mellifera* an exotic bee has an average honey production of 25-40 kg per colony. It is prevalent up to an altitude of 1500 m amsl and used for commercial beekeeping. Both species are excellent pollinators of fruit, oil and field crops and widely used for pollination in high mountain regions. The quality of honey of both the species from different locations of Himachal Pradesh was evaluated under the current scenario.

Materials and Methods

Study area and Sampling

Honey of hive bees i.e *Apis cerana* and *Apis mellifera* was collected from eight selected locations/districts of Himachal Pradesh viz, Solan (L1), Bilaspur (L2), Hamirpur (L3), Lahaul

& Spiti (L4), Kullu (L5), Kangra (L6), Shimla (L7) and Mandi (L8) (Fig.1). Within each district honey was collected from three apiaries or bee keepers (as replications) for *A. cerana* as well as for *A. mellifera*. Collected honey samples were stored at 4±1 °C in refrigerator till further processing.

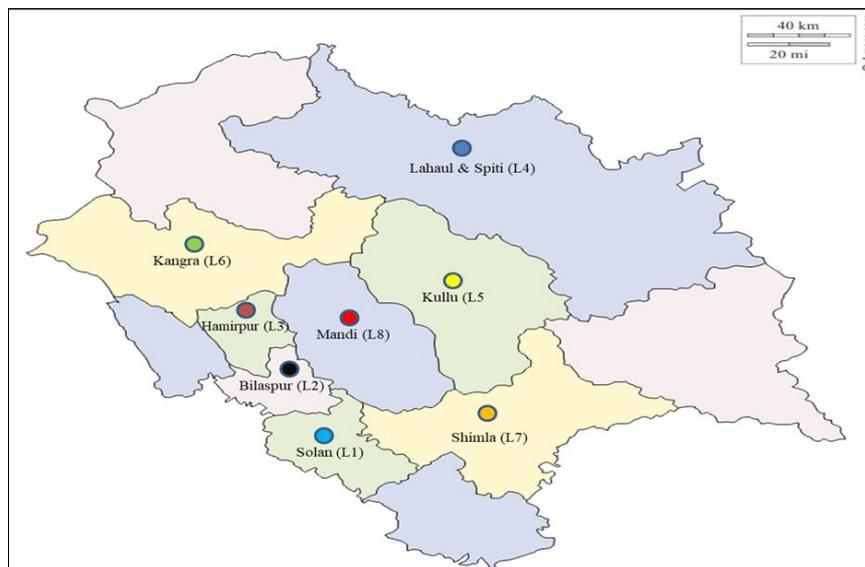


Fig 1: Location map showing different locations of sample collection in Himachal Pradesh

Physical analysis

Physico chemical parameters

Colour

Colour was determined by recording the absorbance of honey (10g) at 560 nm without dilution using spectronic-20 (Bausch and Lomb). Distilled water was used as blank (Townsend, 1969)^[5].

pH

The pH of the honey was measured directly in solution of 10g honey in 100ml distilled water with the help of pH meter (eco Testr pH 2) (AOAC, 2012)^[6].

Moisture

Oven drying method as given by Ranganna (2007)^[7] was used to determine the moisture content.

Sucrose content (g sucrose/g honey)

Sucrose content was determined after inversion of honey solution. Fehling Solution A, Fehling Solution B and Hydrochloric acid (Sp. gr. 1.18 at 20°C) were used to find out the per cent sucrose content. Standard invert sugar solution was prepared by dissolving 0.95g sucrose in 500ml of water followed by the addition of 2ml of concentrated hydrochloric acid and thereafter neutralization with sodium carbonate. The total reducing sugar was calculated as per I.S.I. (1974)^[8].

Glucose and Fructose: Glucose and Fructose content of honey was calculated employing I.S.I. (1974)^[8] method.

Fructose: Glucose ratio: Fructose: Glucose ratio was calculated as per the following formula

Fructose

$$\text{Glucose ratio} = \frac{\text{Fructose (per cent by weight)}}{\text{Glucose (per cent by weight)}}$$

Acidity

Titrimetric method (AOAC, 1984)^[9] was employed to

determine free, lactonic and total acidity of honey. Total acidity was calculated by adding free and lactonic acidities and results were expressed as milli-equivalents of acid per kg of honey.

Total Phenols

The concentration of total phenolic content in honey samples was determined by the Folin-Ciocalteu procedure (Bray and Thorpe, 1954)^[10]. The absorbance of prepared sample solutions was read at 650 nm with the help of spectrophotometer. The concentration of total phenols (mg/100g of honey on fresh weight basis) was determined from the standard curve of catechol.

Hydrogen peroxide

Samples were diluted to a final concentration of 20 per cent (v/v) with ultrapure water (18.2 mΩ cm) and 5 mL of the diluted sample was mixed with 1 mL H₂SO₄ 0.5 M and 5 mL of V₂O₅ (0.2% w/v in 0.5 M H₂SO₄). The absorbance of the solutions at 454 nm was determined using a HACH LANGE DR 5000 UV-visible spectrophotometer. The intensity of the coloured reaction produced in the honey samples is directly related to their level of H₂O₂ production. Quantification of hydrogen peroxide in honey samples was done by comparing the absorbance value with the standards and reading the concentration from the standard curve (Standard addition method) (Pasiak *et al.*, 2018)^[11].

Statistical analysis

The data was analyzed using one-way and two-way analysis of variance (ANOVA) with three replicates after appropriate transformation through online OP-STAT software (Sheoran *et al.*, 1998)^[12] and t-test wherever appropriate. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to classify and discriminate the honey samples and physicochemical parameters of honey through XLSTAT software.

Results and Discussion

Physico-chemical characteristics of *Apis cerana* and *Apis mellifera* honey

pH

The pH value for the *A. cerana* honey ranged from 3.40 to 4.70. Highest pH of 4.70 was recorded for honey of L7 (Shimla), though it was statistically at par with L1 (Solan, 4.50) honey, whereas lowest pH of 3.40 was observed for honey of L5 (Kullu) and L2 (Bilaspur), though both were statistically at par with L4 (lahaul & Spiti, 3.50) and L6 (Kangra, 3.50) honey (Table 1). The pH for the *A. mellifera* honey ranged from 3.60 - 4.80. Highest pH of 4.80 was recorded for honey from L8 (Mandi), which was statistically at par with L5 (kullu, 4.50) honey, whereas lowest pH of 3.60 was observed for honey of L1 (Solan), though statistically at par with L2 (3.80) and L3 (3.70). In the present study, most of the analyzed honey samples from both the bee species were in accordance with the standards for honey set by FSSAI (2018)^[2013] for pH (3.90-6.10). The *A. cerana* honey of L2 (3.40), L4 (3.50), L5 (3.40) and L6 (3.50) and *A. mellifera* honey of L1 (3.60), L2 (3.80) and L3 (3.70) had pH less than 3.90 (lower range of FSSAI standards) indicating acidity towards higher side that enhance fermentation of honey sugar. The present values for pH supports the previous findings of Kamboj and co-workers (2013)^[14] for honey of three major honey producing states of India viz., Punjab, Haryana and Rajasthan where pH was in the range of 3.90 - 4.70 and Saxena and co-workers (2010)^[15], who reported pH value of 3.70 - 4.40 for different Indian honey brands. Irrespective of its geographical origin, honey is generally acidic in nature. The variations in the pH values could be due to honey flow sources (foraged plants), salivary secretions of bees, enzymatic process and fermentative conversion of raw material (Abselami *et al.*, 2018)^[16]. According to Chefrour and co-workers (2009)^[17], honeys with pH range from 3.5 to 4.5 are considered to be blossom honey, while honey with a pH above 5 to be of low quality. Hence the honey of both the hive bees analyzed in the present study can be categorized as blossom honey.

Moisture

The moisture content of *A. cerana* honey varied from 15.70 to 18.60 per cent. Statistically, highest and same moisture content of 18.60 per cent was observed for honey of L7 and L8 followed by L5 (18.00%), which was at par with L4 (17.80%) honey, whereas lowest moisture content of 15.70 per cent was observed for honey of L3 followed by L4 (16.50%), which was in line with L2 (16.66%) honey. The moisture content of *A. mellifera* honey ranged from 15.90 to 18.00 per cent. Statistically, highest moisture content of 18.00 per cent was observed for honey from L8 followed by L5 (17.40%), which was at par with L2 (17.38%) honey, whereas lowest moisture content of 15.90 per cent was observed for honey from L1, which was in line with L4 (16.00%), L6 (16.00%) and L7 (16.00%) honey. The results obtained in the present study are in accordance with the standards set by FSSAI (2018)^[13] for moisture (<20%). Saxena and co-workers (2010)^[15] observed moisture content for honey of commercial honey brands in India well below imposed limit (<20%) set by the FSSAI, which indicated the degree of maturity of honey against fermentation. Also, Kamboj and co-workers (2013)^[14] observed the moisture content of honey from neighbouring states of H.P viz., Punjab, Haryana and Rajasthan varying from 17.08-18.89 per cent. In contrary,

Gairola and co-workers (2013)^[18] observed high values of moisture content (>20%), well above the FSSAI limits for the honey from Uttarkashi district of Uttarakhand, India, ranging from 19.00 to 25.00 indicating extraction of unripe honey (not capped by the bees) from the hives. High moisture content in honey may be due to the extraction of unripe honey (not capped by the bees) from the hives. The variations in moisture content may be due to climatic conditions, botanical origin, processing, storage conditions, degree of maturity in hives, harvesting season and therefore, can vary from year to year (Sahney & Kumar, 2017)^[19]. Higher water content could lead to undesirable honey fermentation during storage giving a bitter taste to honey. More humid conditions before and after honey removal from hives are likely to increase moisture content or vice versa (Imtara *et al.*, 2018)^[20]. Thus, all the investigated honey samples in present study were of good quality as they contained less than 20 per cent water, the maximum amount allowed by FSSAI (2018)^[13].

Colour

The optical density (OD) (absorbance at 560nm) of *A. cerana* honey varied from 0.29 to 1.24. Highest OD of 1.24 was observed for honey from L6 followed by L1 (0.44), though statistically at par with L7 (0.42) and L8 (0.42) honey, whereas lowest OD of 0.29 was observed for honey from L5, though it was statistically at par with L2 (0.36) honey. The optical density of *A. mellifera* honey ranged from 0.33 to 0.66 (Table 4.2). Statistically, highest optical density of 0.66 was observed for honey of L4 followed by L6 (0.51), whereas lowest OD of 0.33 was observed for honey from L1, though statistically in line with L3 (0.35) and L8 (0.39) honey. The honey colour in the present analysis were within the colour designations of honey by White (1975)^[2] i.e within the range of <3.008 (absorbance at 560 nm), according to which the colour variations in the present study ranged from white to light amber for both *A. cerana* and *A. mellifera* honey on the basis of absorbance at 560 nm. Yadav and Satyajeet (2014)^[21] observed extra white, light amber, extra light amber colours for the honey from Ambala, Gurdaspur, lower hills of Kangra, Rohru and Chamba region of India. Also, Adenekan and co-workers (2012)^[22], observed the colour of honey samples from different areas of Ibadan, Oyo State, Nigeria ranged from light amber to dark amber. Variations in the honey colour may be attributed to the botanical origin, composition, storage, processing, rapidity of nectar secretions, management practices, contact with metals, exposure to high temperature and light, etc. Thus, the honey colour may vary from light yellow to amber, dark amber and in extreme cases, it may be black and occasionally, even green or red hues may occur (Khalil *et al.*, 2012)^[23].

Sucrose

The sucrose content of *A. cerana* honey varied from 3.15 to 3.60 per cent. Highest sucrose content of 3.60 per cent was observed for honey from L6, which was in line with L5 (3.47%) and L8 (3.40%) honey, whereas lowest sucrose content of 3.15 per cent was observed for honey from L3, which was statistically at par with L4 (3.17) and L7 (3.30) honey. The sucrose content of *A. mellifera* honey varied from 2.89 to 3.43. Highest sucrose content of 3.43 per cent was observed for honey from L4, which was statistically at par with L1(3.34%) honey, lowest sucrose content of 2.89 per cent was observed for honey from L8, though statistically at par with L3 (2.93%) and L6 (3.00%) honey. All the samples

tested in the present study were in limit (<5%) of sucrose content set by FSSAI (2018) [13]. In accordance with the present study, Saxena and co-workers (2010) [15] found that the average sucrose content in the samples from seven commercial Indian honey brands was 2.8 per cent. Also, Kamal and co-workers (2019) [24] reported the sucrose content of 1.74 to 5.96 per cent in honey samples from northwest Bangladesh. Further, Muli and co-workers (2007) [25] reported that sucrose content of honey was 4.05 per cent and 0.9-2.2 per cent for the Argentinean and Kenyan honeys, respectively. In contrary, the sucrose content observed in the present study is comparatively higher than the reports of Gairola and co-workers (2013) [18], who found sucrose content from 0.19-1.02 per cent for *A. cerana* honey of Uttarkashi district of Uttarakhand. Great variations in sugar composition of honey may be due to the botanical origin, geographical origin, climate, processing and storage (Kamboj *et al.*, 2013) [14].

Sugars are known to change during storage and analysis of sucrose content is very useful to detect the adulteration of honey with table sugar or to check the amount of sucrose naturally found in a given honey sample (Cantarelli *et al.*, 2008) [26]. The probable reasons contributing to higher sucrose content of honey include overfeeding with sucrose syrup, adulteration or premature harvest of honey.

Fructose to glucose ratio

The F: G ratio of *A. cerana* honey varied from 1.06 to 1.73. Highest F: G ratio of 1.73 was observed for honey from L5, though it was statistically at par with L1 (1.70), L2 (1.52), L3 (1.46) and L6 (1.34) honey, whereas lowest F:G ratio of 1.06 was observed for honey from L7, which was statistically at par with L4 (1.26) and L8 (1.18) honey. The F: G ratio of *A. mellifera* honey.

Table 1: Physico-chemical characteristics of *Apis cerana* and *Apis mellifera* honey from different locations of Himachal Pradesh

Locations	Physico-chemical characteristics															
	pH		Moisture (%)		Colour (OD at 560 nm)		Sucrose (%)		F:G ratio		Acidity (meq/kg)		Phenols (mg/100g)		H ₂ O ₂ (mg/kg)	
	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera	A. cerana	A. mellifera
L1 (Solani)	4.50	3.60	17.80	15.90	0.44	0.33	3.17	3.34	1.70	1.44	33.80	39.84	57.90	78.10	186.47	149.31
L2 (Bilaspur)	3.40	3.80	16.66	17.38	0.36	0.43	3.37	3.23	1.52	1.41	35.36	37.86	60.60	101.67	147.82	208.73
L3 (Hamirur)	3.90	3.70	15.70	16.40	0.40	0.35	3.15	2.93	1.46	1.72	31.24	33.23	92.24	95.61	231.21	151.32
L4 (Lahaul & Spiti)	3.50	4.00	16.50	16.00	0.41	0.66	3.17	3.43	1.26	1.09	32.68	36.24	85.51	82.82	272.68	133.26
L5 (Kullu)	3.40	4.50	18.00	17.40	0.29	0.41	3.47	3.18	1.73	1.22	36.63	38.24	99.65	80.80	286.97	167.24
L6 (Kangra)	3.50	4.00	17.20	16.00	1.24	0.51	3.60	3.00	1.34	1.42	38.62	39.26	61.27	63.29	131.93	129.20
L7 (Shimla)	4.70	4.10	18.60	16.00	0.42	0.43	3.30	3.10	1.06	1.38	29.80	36.24	95.61	88.20	168.34	203.42
L8 (Mandi)	4.30	4.80	18.60	18.00	0.42	0.39	3.40	2.89	1.18	1.36	32.22	29.60	81.47	68.68	189.94	122.40
Mean	3.90	4.06	17.38	16.64	0.50	0.44	3.33	3.17	1.41	1.38	33.79	36.31	79.28	82.40	201.92	158.11
CD _(0.05)	0.26	0.30	0.32	0.25	0.07	0.07	0.19	0.16	0.41	0.29	0.49	1.88	5.74	6.47	15.56	14.24

varied from 1.09 to 1.72 (Table 4.2). Highest F: G ratio of 1.72 was observed for honey from L2, though it was statistically at par with L1 (1.44) and L6 (1.42) honey, whereas lowest F: G ratio of 1.09 was observed for honey from L4, which was statistically at par with L5 (1.22), L7 (1.38) and L8 (1.36) honey. The F: G ratio of honey in present study was in accordance with the standard range set by FSSAI (0.95-1.5) for F:G ratio, except L5 (1.73) and L1 (1.70) for *A. cerana* honey and L2 (1.72) for *A. mellifera* honey, indicating slow crystallizing nature of these honey. Present study is supported by Kamboj and co-workers (2013) [14], who observed F:G ratio for the honey from Punjab, Haryana and Rajasthan states of India varying from 0.97-1.23 and Kamal and co-workers (2019) [24], who noticed that the fructose to glucose ratio ranged between 1.14 to 1.34 for the honey of northwest region of Bangladesh. Gairola and co-workers (2013) [18] observed that the honey from Uttarakashi district of Uttarakhand had high F:G ratio upto 2.93. High fructose affects the honey flavor since fructose is much sweeter than glucose (Alvarez-Suarez *et al.*, 2010) [27].

Fructose to glucose ratio is important parameter of honey as it indicates the crystallization state of honey and at high F:G ratio, the honey is fluid (Ouchemoukh *et al.*, 2010) [28]. F/G ratio equal to or less than 1.14 leads to fast growth of honey crystals, whereas ratios greater than 1.3 results in slow growth of honey crystals but proportions higher than 1.58 decreases its value (Escuredo *et al.*, 2014) [29]. Thus, most of the analyzed honey samples in the present study were of slow crystallizing nature.

Acidity

The acidity of the *A. cerana* honey from different locations varied from 29.8 to 38.62 meq/kg. Statistically, highest acidity of 38.62 meq/kg was recorded for honey from L6 followed by L5 (36.63 meq/kg), L2 (35.36 meq/kg) and L1 (33.80 meq/kg) honey, whereas statistically, lowest acidity was recorded for honey from L7 (29.80 meq/kg) followed by L3 (31.24 meq/kg), L8 (32.22 meq/kg), which was in line with L4 (32.68 meq/kg) honey. The acidity of the *A. mellifera* honey varied from 29.6 to 39.84 meq/kg (Table 4.2). Highest acidity was recorded for honey from L1 (39.84 meq/kg), though it was statistically at par with L5 (38.24 meq/kg) and L6 (39.26 meq/kg) honey, whereas statistically, lowest acidity was recorded for honey from L8 (29.60 meq/kg) followed by L3 (33.23 meq/kg). According to the results obtained, none of the analyzed samples exceeded the limit of 50 meq kg⁻¹ as required by the FSSAI Standards for Honey (2018) [13]. Thus, all samples were in compliance with the standards which indicate the freshness of honey samples and absence of honey fermentation. Acidity contributes to honey flavor, stability against microorganisms, enhancement of chemical reactions, antibacterial and antioxidant activity (Gheldorf *et al.*, 2002) [30] and is due to the presence of organic acids, especially gluconic acid, its corresponding lactones, esters and inorganic ions, such as sulfates, phosphates and chlorides. Higher acidity is an indicator of sugar fermentation which is converted into organic acids according to Gomes *et al.*, 2010 [31] and Habib *et al.*, 2014 [32], while low acidity value indicates the freshness of honey samples (Shobham & Nayar, 2017) [33]. In accordance with the present study, Singh and

Bath (1998) [34] stated that the acidity range of Indian honey samples was 29.5 to 41.5 meq/kg. Similarly, Kamboj and co-workers (2013) [14] observed the acidity of North Indian honey ranging from 33.87-37.19 meq/kg. Also, Sodre and co-workers (2002) [35] observed free acidity values ranged between 13.00 to 43.00 meq/kg in honey samples from Northeastern Brazil. Variation in acidity among different honey samples could be due to the different floral origins or may be due to the variation in harvesting seasons (Perez-Arquillue *et al.*, 1994) [36]. Earlier researches showed that high free acidity values may be due to the fermentation of honey sugar by yeasts. In the present study, the acidity values of 29.3 to 38.62 meq/kg for *A. cerana* and 29.6-39.84 meq/kg for *A. mellifera* honey indicated the freshness of honey from different regions of Himachal Pradesh.

Phenols

Total phenol content of *A. cerana* honey varied from 57.90 to 99.65 mg/100g. Statistically, highest phenol content was recorded for honey from L5 (99.65 mg/100g), which was statistically at par with L1 (95.61 mg/100g), whereas lowest phenol content was recorded for honey from L7 (57.90 mg/100g), which was statistically at par with L6 (61.27 mg/100g) and L8 (60.60 mg/100g). The phenol content of *A. mellifera* honey varied from 63.29 to 101.67 mg/100g. Highest phenol content was recorded for honey from L2 (101.67 mg/100g), which was statistically at par with L3 (95.61 mg/100g), whereas lowest phenol content was recorded for honey from L7 (63.29 mg/100g), which was statistically at par with L8 (68.68 mg/100g). Saxena and co-workers (2010) [15] reported phenol content for different Indian honeys varying from 47-98 mg/100g. Similarly, Nayik and Nanda (2015) [37] observed phenol content of 37-117 mg/100g for different unifloral honeys of Kashmir, India. High range of phenolic compounds (158.04-174.87 mg/100g) has been reported for northwestern Bangladeshi honey by Kamal and co-workers (2019) [24], while low values of phenol contents (26.96-70.73 mg/100g) were observed by Imtara and co-workers (2018) [38] for Palestinian honey. Another study by Alvarez-Suarez and co-workers (2018) [39] have noticed an average value for the total phenols in the honey was 54.30 mg/100gm for polyfloral honey samples from Cuba. Phenolic compounds in honey come from nectar, pollen and propolis and may vary according to floral sources (Makawi *et al.*, 2009) [40]. Variations in phenolic compounds in honey is due to beekeeping practices, climatic conditions and biochemical changes in honey constituents (Nayik & Nanda, 2016) [37]. Honey contains large amount of phenolic compounds which have antimicrobial as well as antioxidant properties.

Hydrogen peroxide

The hydrogen peroxide (H₂O₂) content in *A. cerana* honey varied from 131.93-286.97 mg/kg (Table 4.1). Highest H₂O₂ content was observed for honey from L5 (286.97 mg/kg), though statistically at par with L4 (272.68 mg/kg) honey, whereas lowest H₂O₂ content was observed for honey from L6

(131.93 mg/kg) followed by L2 (147.81 mg/kg), L7 (168.34 mg/kg) and L1 (186.47 mg/kg). The H₂O₂ content in *A. mellifera* honey varied from 122.40-208.73 mg/kg (Table 4.2). Highest H₂O₂ content was observed for honey from L2 (208.73 mg/kg), which was statistically at par with L7 (203.42 mg/kg) honey, whereas lowest H₂O₂ content was observed for honey from L8 (122.40 mg/kg), which was statistically at par with L6 (129.20 mg/kg) and L4 (133.26 mg/kg) honey. Present study is supported by Pasi and co-workers (2018) [11], who observed hydrogen peroxide content in the honey samples viz., 301 mg/kg in agros late honey, 412 mg/kg in manuka honey, 280 mg/kg in agros early honey and 184 mg/kg in multifloral honey. Similarly, De Abreu Franchini and co-workers (2007) [41] found H₂O₂ content of 9 to 214 mg/kg in honey from Brazil. The variation in H₂O₂ content and antibacterial activity of honey may be attributed to the different blossoms and herb varieties from where the bees collect nectar and pollen and methods of honey processing (Molan, 1992) [42]. Glucose oxidase and catalase are the main enzymes that are responsible for H₂O₂ production, higher the level of glucose oxidase, higher will be the H₂O₂, lower the catalase level, higher the peroxide level (Dustman, 1979) [43]. During processing of honey, glucose oxidase is degraded leading to the lowering the content of hydrogen peroxide. The information on H₂O₂ content in honey is not available for the honey of Himachal Pradesh.

Comparative physico-chemical characteristics of *Apis cerana* and *Apis mellifera* honey from different locations of Himachal Pradesh

The average pH of *A. cerana* and *A. mellifera* honey was 3.90 and 4.10, respectively (Table 2). No significant difference was observed in the pH of the two species, though it was high for *A. mellifera* honey (4.10) as compared to *A. cerana* honey (3.90). Joshi and co-workers (1999) [44] observed the pH values of 3.62 and 3.52 for *A. cerana* and *A. mellifera* honey, respectively from floristic region in the Chitwan district, central Nepal. Significantly, high moisture content was observed for *A. cerana* honey (17.38%) as compared to *A. mellifera* honey (16.64%). Significantly high moisture content of *A. cerana* honey is also reported by Iftikhar and co-workers (2011) [45], Joshi and co-workers (1999) [44] and Laude and co-workers (1991) [46], which supports the present findings. Moisture content in honey is affected by weather conditions which also persuade the degree of ripeness of honey (Kamboj *et al.*, 2013) [20, 13].

Statistically, highest average optical density of 0.50 was observed in *A. cerana* honey as compared to *A. mellifera* (0.44). The colour of all the samples was white to light amber for both the species. Significantly, highest sucrose content of 3.33 per cent was recorded for *A. cerana* honey as compared to *A. mellifera* honey (3.17%), which is supported by previous findings of Moniruzzaman and co-workers (2013) [47], who observed the mean sucrose content of *A. mellifera* honey samples (3.34%) was lower than that of *A. cerana* honey (3.74%).

Table 2: Comparative physico-chemical characteristics of *Apis cerana* and *Apis mellifera* honey from different locations of Himachal Pradesh

Species	Physico-chemical characteristics							
	pH	Moisture (%)	Colour (OD at 560nm)	Sucrose (%)	F:G ratio	Acidity (meq/kg)	Phenols (mg/100g)	H ₂ O ₂ (mg/kg)
<i>Apis cerana</i>	3.90	17.38	0.50	3.33	1.41	33.8	79.28	201.92
<i>Apis mellifera</i>	4.10	16.64	0.44	3.17	1.38	36.32	82.40	158.11
t(0.05)	2.50	14.39	5.65	12.09	0.71	11.22	4.40	12.43

The average F:G ratio of *A. cerana* and *A. mellifera* honey was 1.41 and 1.38, respectively. No significant difference was

observed in the F:G ratio of the two species in the present study. Joshi and co-workers (1999) [44] also observed no

significant difference in F:G ratio for *A. mellifera* (1.10) and *A. cerana* (1.11) honey. The average acidity of *A. mellifera* honey (36.32 meq/kg) was statistically higher than *A. cerana* honey (33.78 meq/kg). Significant difference was observed in the phenol and hydrogen peroxide content of the two species. Significantly, highest phenol content was observed in *A. mellifera* honey (82.40 mg/100g) as compared to *A. cerana* honey (79.28 mg/100g), whereas hydrogen peroxide content was highest in *A. cerana* honey (201.92 mg/kg) as compared to *A. mellifera* (158.11 mg/kg). In *A. cerana* honey, moisture content, OD, sucrose content, F:G ratio and H₂O₂ content were found highest, though significant difference was recorded only for moisture content, OD, sucrose content and H₂O₂. Similarly, in *A. mellifera* honey, pH, acidity and phenols were found to be highest, though statistical difference was recorded only for acidity and phenols.

2.3. Principal component analysis

Principal component analysis (PCA) is commonly used to examine the relationship between data and samples along with their distribution. Moreover, PCA is recognized to be a valuable tool for the abstraction of important information from a multivariate matrix (Imtara *et al.*, 2018)^[20]. The PCA analysis concluded that first three principal components

contributed 77.33 per cent of the total variance in the physico-chemical properties of the examined honey. PC1, PC2 and PC3 contributed 32.46, 26.34 and 18.53 per cent, respectively of the total variance with Eigen values greater than 1.0 (2.59 for PC1, 2.10 for PC2 and 1.48 for PC3). The first principal component (PC1) mostly controlled by the characters such as pH, F:G ratio and phenols which were elucidated 32.46 per cent of the variance. However, the second principal component clarified 26.34 per cent of the variance subjected by colour, sucrose and acidity fructose to glucose ratio. Additionally, the third principal component (PC3) scrutinized more than 18.53 per cent of the variance governed by moisture and hydrogen peroxide. These results concluded that all the evaluated parameters in the present study could be used to categorize the honey samples. Referring to the PCA biplot (Fig. 2a, 2b), analyzed honey samples of hive bees were discriminated successfully.

Table 3a: Principal component analysis

	PC1	PC2	PC3
Eigen value	2.597	2.107	1.482
Variability (%)	32.461	26.343	18.531
Cumulative %	32.461	58.804	77.335

Table 3b: Principal component analysis

Physico-chemical parameters	Principal components		
	PC1	PC2	PC3
pH	0.503	0.183	0.073
Moisture	0.205	0.036	0.534
Colour	0.044	0.677	0.000
Sucrose	0.004	0.499	0.351
Fructose:Glucose ratio	0.634	0.043	0.000
Acidity	0.289	0.413	0.047
Phenols	0.735	0.100	0.008
H ₂ O ₂	0.184	0.158	0.469

*Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Based on PCA, Kamal and co-workers (2019) concluded that the first three principal components i.e PC1, PC2 and PC3 were accounted for 58.11, 17.79 and 13.19 per cent of the total variance (89.08%) with Eigen values (>1) 11.62, 3.56 and 2.64, respectively (Table 3a). The first principal component (PC1) frequently governed by the moisture content, total soluble solids, total solids, pH, HMF, reducing sugar, fructose, glucose, diastase activity, total phenols, flavonoids and L* color coordinates (Table 3b).

In present study, eight analyzed parameters of total sixteen honey samples was inputted into STAT as variables, between group average linkage method was applied to sort honey types into groups, and rescaled distance was selected as measurement to obtain a hierarchical cluster analysis (HCA) dendrogram shown in Fig. 3.

The samples can be divided into two main groups. The first cluster can be divided into three sub-groups as, sub-group A: *A. mellifera* honey from Kangra, Lahaul&Spiti, Solan and *A. cerana* honey from Bilaspur; sub-group B: *A. mellifera* honey from Hamirpur, Bilaspur and *A. cerana* honey from Solan; sub-group C: *A. cerana* honey from Hamirpur, Lahaul &Spiti and Kullu. Similarly, second cluster can be divided into 2 sub-groups as, sub-group D: *A. cerana* honey from Kangra; sub-group E: *A. mellifera* honey from Mandi, Kullu, Shimla and *A. cerana* honey from Shimla and Mandi. The HCA successfully clustered honey samples together with the maximum similarities. Kivrak & co-workers (2017)^[48], while categorized Turkish honeys into three groups with HCA. First

cluster was divided into three sub-groups including cedar, linden, pine, multiflower, lavender, carob, chestnut, rhododendron, vitex, sunflower, heather honeys. Second cluster included Cedar honeys with high HMF as well as eucalyptus, clover, citrus, cotton and acacia honeys. The third cluster included thyme and sideritis honeys.

Conclusions

The analysis of various physico-chemical parameters *viz.*, pH, moisture, colour, sucrose content, F:G ratio, acidity, phenols and hydrogen peroxide content in the present study concluded that the honey from hive bees (*A. cerana* and *A. mellifera*) of Himachal Pradesh is of good quality as most of the analyzed parameters were in the range of approved limits by FSSAI. Additionally, the analyzed honey samples met the required standards of Indian and International markets, justifying the suitability of hive bees honey for export purpose. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) successfully classified and discriminated the analyzed physico-chemical parameters of honey samples from different hive bee species as well from different locations.

Acknowledgments

The authors would like to acknowledge Dr. YSP University of Horticulture and Forestry Nauni, Solan for financial support and beekeepers of Himachal Pradesh for supplying the honey samples for investigation.

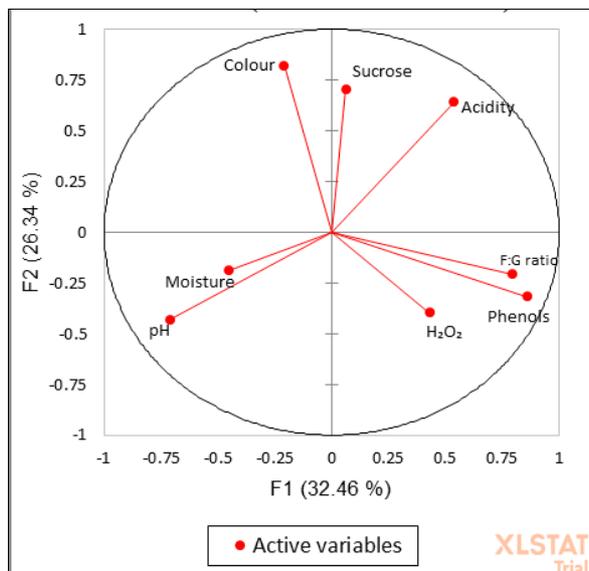


Fig 2a: Variables (axes F1 and F2: 58.80 %)

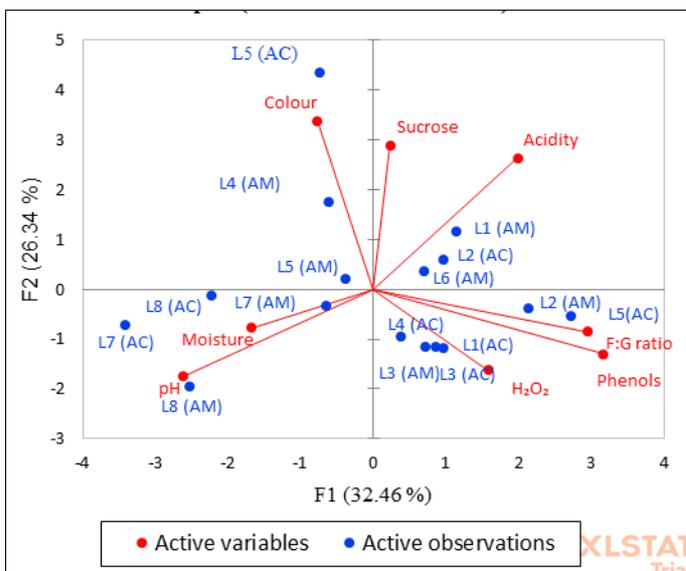


Fig 2b: Biplot (axes F1 and F2: 58.80 %)

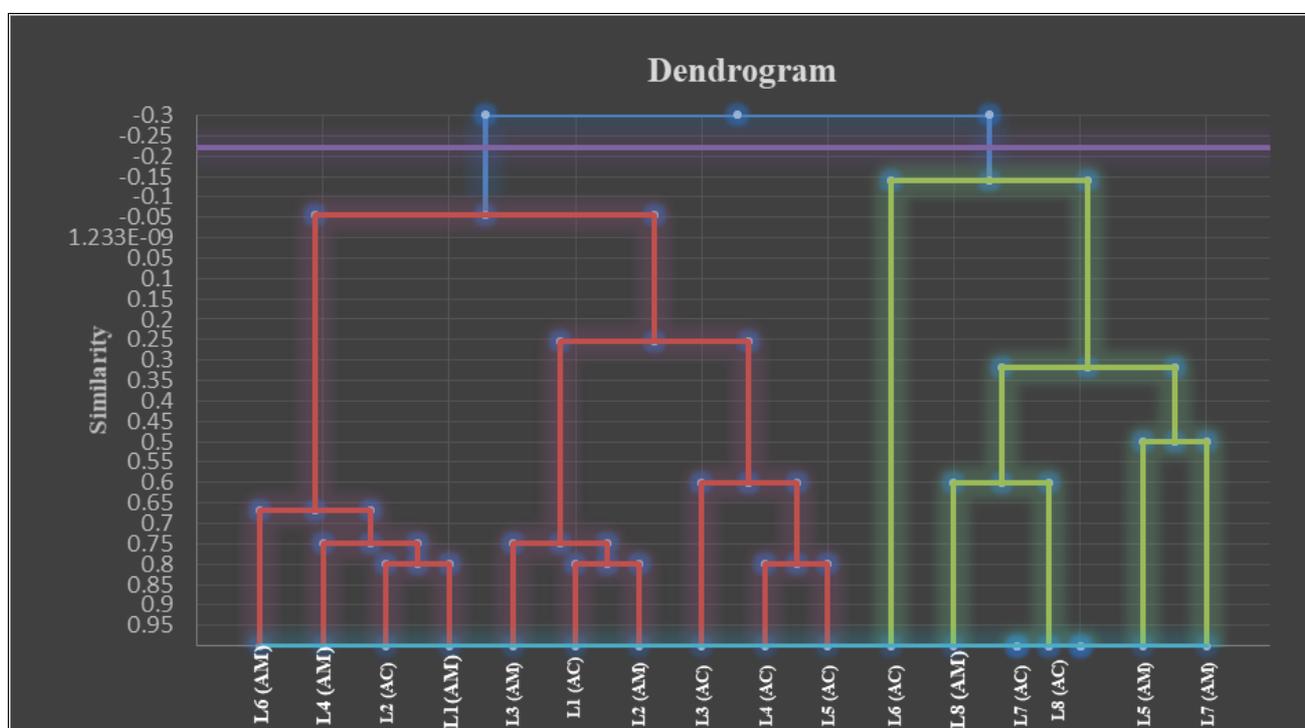


Fig 3: Principal component analysis (PCA) of the analyzed characteristics of *Apis cerana* (AC) and *Apis mellifera* (AM) honey: a) PCA of the physico-chemical characteristics of honey; b) Distribution of honey samples conferring to PCA: Solan (L1), Bilaspur (L2), Hamirpur (L3), Lahaul & Spiti (L4), Kullu (L5), Kangra (L6), Shimla (L7) and Mandi (L8); c) Hierarchical cluster analysis dendrogram of two honey bee species i.e. *A. cerana* (AC) and *A. mellifera* (AM) from eight different locations.

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