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Physico-Chemical and Antioxidant Evaluation of Honey Collected from Three Different Honeybee Species in Chamba District, Himachal Pradesh, India

Sharma Ankush[1*], Kumar Rajesh[2], Singh Chander Shemsher[1], Dogra Nizul[1], Kumari Shikha[1] and Kuthleria Kavita[1]

[1]Department of Zoology, Sri Sai University Palampur, Kangra, Himachal Pradesh, India
[2]Department of Biosciences, Himachal Pradesh University, Shimla, Himachal Pradesh 175001, India

**Corresponding Author*

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Abstract: Honey samples were obtained from three different species i.e., *Apis cerana*, *Apis dorsata* and *Apis mellifera*, in stored and fresh conditions and examined for their physio-chemical as well as antioxidant properties. It is the first attempt to compare the physico-chemical and antioxidant properties of honey samples obtained from three different species of honeybees (stored and fresh samples) of the Chamba region. The stored honey sample of *Apis mellifera* represented the highest pH, acidity, TDS, and moisture content, but showed the least specific gravity, while the fresh honey sample of *Apis cerana* showed the highest specific gravity, electrical conductivity, and antioxidant value. A fresh sample of *Apis dorsata* showed the highest optical density and ash content. Other values of honey samples (fresh and stored) for all three species were compared with previous reports published by different authors in different parts of the world and found within satisfactory limits.

Keywords: Honey, Strains, Chamba, Physico-chemical, Antioxidant

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Introduction

Honey is a sugary and clammy substance produced by honey bees. These bees hover over flowers and suck the nectar which contains reducing and non-reducing sugars (Jonathan and White, 1978; Azeredo *et al*., 2003; Kumar *et al*., 2022). Honey possesses wonderful therapeutic potential and is used to cure many diseases such as eye disease, fatigue, tuberculosis, throat infection, ulcers, diabetes, cough and cold etc. (Samarghandian and Farkhondeh, 2017). In ancient Egypt, it was used for mummification (Shariquie and Najim, 2004). Honey plays a major role in the field of Ayurveda for the last 4000 years.

Honey is composed of about 80-85% carbohydrates mainly glucose or fructose, 0.1 - 0.4% proteins, 15-17% water, 0.2% ash and a smaller amount of other substances (Balos *et al*., 2019). Honey can be used to treat a wide range of wounds including pressure ulcers, diabetic foot ulcers, infected wounds resulting from sugary or injury and burns. Honey is a natural substance, effective in the bacterial growth inhibition and help in the treatment of a wide range of wound types, including burns, scratches, diabetic boils (Skin abscesses associated with diabetic), malignancies, leprosy, fistulas, leg ulcers, traumatic boils, cervical and varicose ulcers, amputation, burst abdominal wounds, septic and surgical wounds, cracked nipples, and wounds in the abdominal wall (Tashkandi, 2021). The composition of honey is beneficial for the treatment of diarrhoea due to the presence of probiotics, prebiotics and Zinc (Hussain, 2018). It has been noted that its components and antioxidant capacity depend on the product's origin, source of flowers, season and environment as well as the manufacturing process (Mracevic *et al*., 2020). Cummings and Stephen (2007) reported that the sugar which is present in honey is not of single type but it contains 3 kinds of sugar -- fructose (fruit sugar) is about 41%; glucose (grape sugar) about 31% and the rest of the sugar is sucrose (ordinary sugar) is about 1 to 3%. Sharma *et al.* (2021) also compared the honey obtained from different origins in the Kangra region. There are limited studies on the physio-chemical and antioxidant properties of honey obtained from three different honeybee species in Himachal Pradesh (Shivani *et al*., 2021). No records are available on the comparison of fresh and stored honey samples obtained from the three different honeybee species of district Chamba. Keeping in view the above researches, the present study was designed to investigate the quality of stored and fresh honey produced by *A. cerana*, *A. dorsata* and *A. mellifera.*

Materials and Methods

Sampling, Filtration and Storage:

Three different locations in district Chamba (H.P.) were selected for the sampling of honey. Six samples of three different species of honey bees (one fresh and one stored) were collected from each location. Samples were heated at 40 ℃ for almost 30 min. Samples were filtered in the laboratory by using a filter mess made of cotton cloth and then kept for 24 h. Honey samples filtered in the laboratory were finally stored in 500 ml wide mouth properly labelled glass bottles at room temperature ranging from 20 to 35 ℃ till further use.

Analysis of Physico-Chemical and Antioxidant Properties:

The honey colour analysis was performed using the Pfund scale (Marchini *et al.*, 2004; Biochrom, 2013). pH was directly measured from a pH Meter (Noori *et al*., 2012). Moisture content was measured by the procedure described in Codex Alimentarius (2001). Specific Gravity was measured by the technique described by Noori *et al.* (2012). In order to find out Acidity, Electrical conductivity, optical density and ash content methods described by AOAC (1990) were followed. TDS was directly measured by TDS meter. For D.P.P.H. radical scavenging activity, 0.75ml of the sample was mixed with 0.75 ml of 0.1 mM of DPPH in methanol. The measurement of radical scavenging activity was done using Trolox, BHT as standard and the value is expressed as CS50 (mg/ml), the concentration of the sample that 50% scavenging of DPPH radical.

Results and Discussion

The results of analysed honey samples are shown in Tables 1 and 2. Most of the samples were Amber in colour. The fresh honey sample of *A. cerana* showed light amber colour, while the stored sample was Amber in colour. The fresh honey sample of *A. dorsata* showed an extra light colour and the stored samples showed dark Amber, while a fresh sample of *A. mellifera* showed an Amber colour and the stored sample showed white amber colour. Honey colour is formed exclusively by the carotenoid, xanthophyll, and flavonoid coloured

Table 1: The average value of Physical parameters of honey of three different bee species (*Apis cerana*, *Apis dorsata* and *Apis mellifera*)

S. No.	Parameters	*A. cerana*		*A. dorsata*		*A. mellifera*	
		Fresh	**Stored**	**Fresh**	**Stored**	**Fresh**	**Stored**
1	Colour	Light Amber	Amber	Amber	Dark Amber	Extra white	Amber
2	Moisture	24.2%	27.3%	24.56%	19.6%	18%	28.92%
3	Specific Gravity	1.22 g/cm	1.21 g/cm	1.17 g/cm	1.21 g/cm	1.20 g/cm	1.17 g/cm
4	Electrical Conductivity	0.708 mS/cm	0.400 mS/cm	0.108 mS/cm	0.396 mS/cm	0.159 mS/cm	0.139 mS/cm
5	Optical Density	0.112	0.083	0.118	0.020	0.105	0.074
6	Ash Content	0.02%	0.06%	0.20%	0.79%	0.04%	0.05%

pigments present in the nectar and the pollen collected by the bee (Pérez-Pérez *et al*., 2012). The honey which is darker in colour is stronger and honey which is lighter in colour is a mild flavour in taste as observed by Sharma *et al.* (2023). The highest pH observed in the stored honey sample of *Apis mellifera* was 4.7, while the fresh honey sample showed 4.5 pH. In *Apis cerana* stored sample pH was 4.2, while the fresh sample showed pH 4.6. In *Apis dorsata*, the pH of fresh honey was 4.4 and the pH of stored honey was 4.3. The acceptable range of pH of honey is between 3.2 to 4.5 as reported by Khalil *et al.* (2012). The highly acidic *A. mellifera* honey samples indicate the possible fermentation of sweetness (Sugars) into organic acids (Ibrshim, 2012).

The Moisture content of the fresh honey sample was analysed and found 24.2% in the *Apis cerana* and in stored honey 27.3%. The moisture of the fresh honey sample collected from the *Apis dorsata* was 24.57% and in stored honey moisture content was 19.6%. The least moisture analysed in *Apis mellifera* was 18% and the stored honey sample of *Apis mellifera* had the highest moisture content 27.90%. According to Buawangpong and Burgett (2019), the average honey moisture from only one species *Apis mellifera* falls below the mandated 20% although *Apis cerana* and *Apis dorsata* exceed 20% at levels of 21.2% and 22.7%, respectively. The value of moisture content in the honey samples lies in between 18.6% and 29.87% as reported by Khalil *et al.* (2012).

The average specific gravity of fresh honey sample of *Apis cerana* was 1.22 g/cm and stored honey was 1.21 g/cm. The value of specific gravity of fresh honey of *Apis dorsata* was 1.179 g/cm and of stored honey was 1.201 g/cm. The average value of specific gravity of fresh honey of *Apis mellifera* was 1.202 g/cm and of the stored honey was 1.174 g/cm. The *A. mellifera* honey has maximum 1.38 g/cm specific gravity while the *A. dorsata* honey has 1.36 g/cm and *A. cerana* has a 1.38 g/cm specific gravity observed by the Krishnashree and Ukkuru (2017) which is in agreement with the present study. The specific gravity of honey depends upon the water content, temperature and solid substances (Albu *et al*., 2021). The honey sample was analysed for acidity and found that in *Apis cerana* honey, the fresh sample showed 0.28% acidity the and stored sample showed 0.27%, while *Apis dorsata* honey acidity was measured 0.19% in the stored sample and 0.25% in the fresh sample. The highest acidity was recorded in the stored honey sample of *Apis mellifera* is 0.33% and lowest acidity was noted in the fresh sample of *Apis mellifera* i.e. 0.11%.

Higher acidity value indicates the sugar fermentation result in the formation of organic acids (Gomes *et al.*, 2010; Habib *et al.*, 2014), while low acidity value indicates the freshness of honey samples (Shobham and Nayar, 2017). Singh and Bath (1997) explained that acidity is the number of acidic characters in the sample of honey. The Average electrical conductivity of fresh *Apis dorsata* sample was 0.108 mS/cm and the stored sample showed 0.396 mS/cm. The highest Electrical Conductivity was observed in fresh *Apis cerana* as 0.708 mS/cm, while the stored sample showed 0.400 mS/cm and the average Electrical Conductivity of stored samples of *Apis mellifera wa*s 0.135 mS/cm, while fresh sample have and 0.158 mS/cm. According to Živkov-Balos *et al.*(2018) the electrical conductivity is closely related to the presence of concentration of minerals and organic acid, the value of electrical conductivity in the investigated honey samples was between 0.08 and 1.99 mS/cm. The present study showed some variations in results because electrical conductivity is depending upon the flora and pollen content of that particular area in honey as explained by Khalil *et al.* (2012).

The optical density of different honey samples was measured with the help of a Spectrophotometer. The optical density observed in the stored sample of *Apis dorsata* was 0.020 and in the fresh sample it was 0.118. The Optical density observed in the fresh sample of *Apis mellifera* was 0.105, while 0.074 in the stored sample. Optical density was observed in the fresh sample of *A. cerana* was 0.112 and Optical density 0.083 was observed in the stored sample of *A. cerana.* The optical density of honey is mainly affected by the nectar and pollen present in it. Optical density is an important factor that helps in determining the freshness and the colour of honey. The optical density values increase or decrease with the increase in the moisture content of honey. Moulya (2023) reported the optical density of *Apis cerana* from Chikkamagaluru as 0.110. The optical density of the honey sample obtained from *Apis dorsata* is 0.218 and 0.186 is the optical density of *Apis mellifera* reported by Louveaux *et al.* (1978). The above observations show that the TDS of the fresh honey sample of *Apis cerana* was 228 ppm and 169 ppm TDS was recorded in the stored sample. While the TDS of the fresh honey sample of *Apis dorsata* was 411 ppm and the stored sample showed 205 ppm. The TDS of the stored sample of *Apis mellifera* was 475.5 ppm while the fresh sample showed 378 ppm. TDS is the total amount of mobile charged ions including minerals, salt or metals dissolved in a given volume of water. According to Moniruzzaman *et al.* (2013), the TDS value of *A. cerana* was comparatively higher 494.33 ppm as compared to the *A. dorsata* 371 ppm and *A. mellifera* 375 ppm. TDS showed the presence of inorganic and organic substances present in honey while the higher value of TDS in the honey is due to the substances such as micro granular (AOAC, 1990).

The highest Ash content observed in the stored sample of *A. dorsata* was 0.79% while the fresh sample showed 0.20%. The least Ash content obtained from the fresh sample of *A. cerana* was 0.02% and the stored sample showed 0.06%. The ash content in the fresh sample of *A. mellifera* was observed as 0.04% and in the stored sample as 0.05 %. According to Gairola *et al.* (2013), the ash content of *A. cerana* ranged between 0. 09 to 0.18% which is higher than present study. According to Kamal *et al.* (2002) high value of ash contents may also depend upon the floral origin of honey and the material collected by bees. Ash content is one of the most important quality criteria for estimating the origin of honey (Anupama *et al.,* 2023).

The highest DPPH radical scavenging activities were observed in the fresh sample of honey of *Apis cerana* 60.38 %, while the stored sample showed 48.72%. The least DPPH antioxidant activity was observed in the stored honey sample of *Apis dorsata* which was 48.98% and the fresh sample showed an average DPPH as 51.91%. The fresh honey sample of *Apis mellifera* showed 51.91%

Table 2: The average value of Chemical and Antioxidant parameters of honey of three different bee species (*Apis cerana*, *Apis dorsata* and *Apis mellifera*)

S. No.	Parameters	*B. cerana*		*A. dorsata*		*B. mellifera*	
		Fresh	Stored	Fresh	Stored	Fresh	Stored
1	pH	4.6	4.2	4.2	4.3	4.5	4.7
2	Acidity	0.28%	0.27%	0.25%	0.22%	0.14%	0.37%
3	T.D.S.	228 ppm	169 ppm	411 ppm	205 ppm	378 ppm	475.5 ppm
4	D.P.P.H	60.38%	48.72%	51.91%	40.98%	49%	55.87%

DPPH antioxidant activity in honey, while the stored sample showed 55.87%. Nayaka *et al.* (2020) reported that the DPPH of *A. cerana* has a value from the 33.52% to 72.89 % and has antioxidant activities on a large scale. While 25.94% to 53.89% DPPH in *A. dorsata* was observed by Krishnasree and Ukkure (2016). The DPPH of *A. mellifera* was 29.42 % to 59.89% (Nascimento *et al*., 2018).

Conclusion

The Physio-chemical and antioxidant properties of stored and fresh honey samples obtained from three different honeybee species i.e., *A. cerana*, *A. dorsata* and *A. mellifera* of district Chamba (Himachal Pradesh) were analysed, compared and found that the values of all parameters were within the Codex standard for honey (Alimentarius, 2001). Although all the parameters of different samples showed minor variations in their results, both fresh and stored samples can be considered of good quality.

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References

Albu A, Radu-Rusu, CG, Pop IM, Frunza G and Nacu G. (2021) Quality assessment of raw honey issued from eastern Romania. Agriculture 11(3): 247.

Alimentarius C. (2001) Codex standard for sugars (Codex Stan 212-1999). Practice: 1-5.

Anupma D, Bhatt KK and Sapna VK. (2003) Sensory and physico-chemical properties of commercial samples of honey. Food Res Int. 36: 183-191.

AOAC (1990) Association of official analytical chemists. Official methods of analysis. 15th edition Arlington, VA, USA.16: 613-619.

Baloš MŽ, Jakšić S, Popov N, Mihaljev Ž and Pelić DL. (2019) Comparative study of water content in honey produced in different years. Arch Vet Med. 12(1): 43-53.

Biochrom (2013) Measurement of Honey Quality. [Serial [3]Online]. www.biochrom.co.uk.

Buawangpong N and Burgett M. (2019) Capped honey moisture content from four honey bee species; *Apis dorsata* F., *Apis florea* F., *Apis cerana* F, *and Apis mellifera* L. (Hymenoptera: Apidae*)* in Northern Thailand. J Apicult. 34(2): 157-160.

Cummings JH and Stephen AM. (2007) Carbohydrate terminology and classification. European J Clin Nutr. 61(1): S5-S18.

Da C Azeredo L, Azeredo MAA, De Souza SR and Dutra VML. (2003) Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins. Food Chem. 80(2): 249-254.

Do Nascimento KS, Sattler JAG, Macedo LFL, González CVS, de Melo ILP, da Silva Araújo E, Granato D, Sattler A and de Almeida-Muradian LB. (2018) Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian *Apis mellifera* honeys. LWT. 91: 85-94.

Gairola A, Tiwari P and Tiwari JK. (2013) Physico-chemical properties of *Apis cerana-indica* F. honey from Uttarkashi district of Uttarakhand, India. J Global Biosci. 2(1): 20-25.

Gomes S, Dias LG, Moreira LL, Rodrigues P and Estevinho L. (2010) Physicochemical, microbiological and antimicrobial properties of

commercial honeys from Portugal. Food Chem Toxicol. 48: 544-548.

Habib HM, Al Meqbali FT, Kamal H, Souka UD and Ibrahim WH. (2014) Physicochemical and biochemical properties of honeys from arid regions. Food Chem. 153: 35-43.

Hussain MB (2018) Role of honey in topical and systemic bacterial infections. J Altern Complement Med. 24 (1): 15-24.

Jonathan W and White J. (1978) Agricultural research service, U.S. Department of Agriculture Philadelphia, Pennsylvania. Adv Food Res. 24: 287-374.

Kamal A, Saeeda R, Nouman N, Tabassum N, Musarrat G, Qurehi M and Nasim K. (2002) Comparative study of honey collected from different flora of Pakistan. Onl J Biol Sci. 2(9): 626-627.

Khalil MI, Moniruzzaman M, Boukraâ L, Benhanifia M, Islam MA, Islam MN, Sulaiman SA and Gan SH. (2012) Physicochemical and antioxidant properties of Algerian honey. Molecules 17(9): 11199-11215.

Krishnasree V and Ukkuru PM. (2017) Quality analysis of bee honeys. Int J Curr Microbiol Appl Sci. 6(2): 626-636.

Kumar R, Agrawal OP and Hajam YA (2022) Honey: A Miraculous Product of Nature. CRC, Taylor Francis, New York.

Louveaux J, Maurizio A and Vorwohl G. (1978) Methods of Melissopalynology. Bee World 59: 139-157.

Marchini LC, Moreti ACCC and Otsuk IP. (2005) Análise de agrupamento, com base na composição físico-química, de amostras de méis produzidos por *Apis mellifera* L. no estado de São Paulo [Cluster analysis, based on the physical and chemical composition of honey samples produced by *Apis mellifera* L. in São Paulo, in Portuguese]. Ciência e Tecnologia de Alimentos 25: 8-17.

Moniruzzaman M, Sulaiman SA, Khalil MI and Gan SH. (2013) Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with *manuka* honey. Chem Central J. 7: 1-12.

Moulya MR. (2023) Physical properties of the stingless bee honey in comparison with *Apis cerana* honey samples collected from different geographical locations of Karnataka and Kerala. Pharma Innov J. 12(5): 3028-3034.

Mračević SĐ, Krstić M, Lolić A and Ražić S. (2020) Comparative study of the chemical composition and biological potential of honey from different regions of Serbia. Microchem J. 152: 104420.

Nayaka NMD, Fidrianny I, Hartati R and Singh M. (2020) Antioxidant and antibacterial activities of multiflora honey extracts from the Indonesian *Apis cerana* bee. J Taibah Univ Med Sci. 15(3): 211-217.

Noori Al-Waili, Khelod Salom, Ahmed Al-Ghamdi and Mohammad JA. (2012) Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. Scientific World J. 2012: 930849.

Pérez-Pérez E, Vit P, Rivas E, Sciortino R, Sosa A, Tejada D and Rodríguez-Malaver AJ. (2012) Antioxidant activity of four color fractions of bee pollen from Mérida Venezuela. Arch Latinoam Nutr. 62(4): 375-380.

Samarghandian S, Farkhondeh T and Samini F. (2017) Honey and health: A review of recent clinical research. Pharmacogn Res. 9(2): 121.

Sharma A, Rana S, Ruchika, Sharma P and Sharma M. (2021) Evaluation of physiochemical properties of honey of *Apis mellifera* (Himachal Pradesh): A comparison with *Apis dorsata.* Biol Forum 13(1): 181-185.

Sharma R, Thakur M, Rana K, Devi D and Bajiya MR. (2023) Honey, its quality and composition and their responsible factors. Int J Bio-resource Stress Managem. 14(1): 178-189.

Sharquie KE and Najim RA. (2004) Embalming with honey. Saudi Med J. 25(11): 1755-1756.

Shivani, Hajam Younis Ahmad and Kumar R. (2021) Physico-chemical analysis of honey samples collected from *Apis cerana* in Western Himalayan region. J Entomol Res. 45(2): 246-253.

Shobham KKC and Nayar J. (2017) Physicochemical analysis of some commercial honey samples from Telangana. Indian J Nutr. 4: 153.

Singh N and Bath KP. (1997) Quality evaluation of different types of Indian honey. Food Chem. 58: 120-133.

Tashkandi H. (2021) Honey in wound healing: Updated review. Open Life Sci. 16(1): 1091-1100.

Živkov-Baloš M, Popov N, Vidaković S, Ljubojević-Pelić D, Pelić M, Mihaljev Ž and Jakšić S. (2018) Electrical conductivity and acidity of honey. Arch Vet Med. 11(1): 91-101.