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Evaluation of Physiochemical Properties of Honey of *Apis mellifera* (Himachal Pradesh): A Comparison with *Apis dorsata*

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ABSTRACT: Evaluation of honey quality is an important area of research which aimed at preventing falsification and likewise the physiochemical data of honey is desired for its proper storage and marketing. The present study aimed to analyze physiochemical properties of honey from *Apis mellifera* and *Apis dorsata*. Physical parameters such as pH, moisture content, electrical conductivity, acidity, specific gravity, ash content, color range and colour analysis were determined. Biochemical parameters in terms of total carbohydrates, total reducing sugar, reducing sugar, fructose, sucrose, glucose, fructose/glucose ratio were examined. Significant differences ($p<0.05$ and $p<0.001$) were remarked in physiochemical properties. This was our first attempt to compare the properties of honey obtained from Nagrota Bagwan bee and wild origin bee. The results were comparable with reports from many parts of the world and also within the limits of international standards.

Keywords: *Apis mellifera; Apis dorsata;* Physiochemical analysis; Quality; Storage

INTRODUCTION

Honey is a pure natural liquid mixture of sugars (mainly glucose and fructose) (Nascimentoa *et al.*, 2018), protein, enzymes, phenolic compound, amino acid, vitamins, ascorbic acid, organic acids and several minerals like calcium, potassium, phosphorous, magnesium, iron and sodium and is also beneficial in balanced biological processes(Vilhena and Almeida-Muradian, 1999; Habib *et al.*, 2014; Das *et al.*, 2015).

The International Honey Commission (IHC) has proposed few physiochemical parameters which include pH, moisture content, ash content, colour analysis, carbohydrates, sugars (glucose, sucrose, fructose) (Bogdanov *et al.*, 1999). Physiochemical analysis is an important criterion for storage, granulation, flavor, nutritional, medicinal quality and also for marketing purposes (Attri, 2011).

The composition of honey depends upon plant species, geographical location, climatic condition, soil characterization, different minerals present in soil and environmental storage condition (Bertoncelj *et al.*, 2007; Kaur *et al.*, 2016; Vijayakumar *et al.*, 2020). To the best of our knowledge, although there are some sort of reports on the physiochemical properties of honey produced in Nagrota Bagwan region of Himachal Pradesh but there is a limitation of a scientific research on the quality of honey produced. Keeping in mind, present study was designed to investigate the quality of honey produced by *A. mellifera* and compare it with *A. dorsata* honey from same region/location.

MATERIALS AND METHODS

Honey samples and storage: A composite honey sample of *A. mellifera* was purchased directly from the farm gates of Bee-keeping research station, Nagrota Bagwan, Himachal Pradesh in December and *A. dorsata* sample was obtained from a local distributor and were stored at room temperature (22–24°C) in air tight plastic containers till further analysis.

Physical parameters: The pH was read directly from pH meter (Bogdanov, 2009). In order to determine acidity and specific gravity modified method of FSSAI (2015) was followed. The electrical conductance of the specified solution was measured using the calibrated digital conductivity meter (Bogdanov *et al.*, 1999). The moisture content of honey was deliberated in triplicate via measuring the refractive index of the sample at 20 ^{o}C (Bogdanov *et al.*, 1997; AOAC, 2012). The ash content of the honey samples was analysed by following the procedure of QSAE (2005).

The honey color analysis was performed using the Pfund scale (Marchini *et al.*, 2004; Biochrom, 2013). In addition, colour was also determined by spectrophotometric measurement as described by Beretta *et al.* (2005). Optical density was measured by the modified method of AOAC (1999) and measured with the help of spectrophotometer at 660 nm

Biochemical parameters: Total carbohydrate content in honey samples was determined using phenol reagent and yellowish brown color developed was read at 490 nm in a spectrophotometer (Dubois *et al.*, 1956). Total

reducing sugar content of the honey samples was determined by the modified procedure described by FSSAI (2015). The reducing sugar content of the honey samples were studied by using Fehling's test (Lane and Eynon modified method). The Fructose, glucose, sucrose content and Fructose: Glucose ratio of the honey samples was determined following the modified procedure of AOAC (1999).

Fiehe's Test and Aniline Chloride Test (ACM): Both were done by the modified method of FSSAI (2015).

Statistical Analysis: The results of all experiments were expressed as mean±SD of triplicate measurements. The significant differences were represented by students T-test and were calculated using SPSS variants 22 (TBM, corporation New York, USA) and Microsoft office excel 2007.

RESULTS AND DISCUSSION

Physical parameters: Results of analysed honey samples were shown in table 1.

Analysis of pH and acidity of honey is important because it affects the quality, stability, texture and shelf life of honey. The pH for *A. mellifera* was 4.16±0.06 and for *A. dorsata* was 4.43±0.21 while acidity was found to be 25.67±1.15mEqKg^{-1} (*A. mellifera*) and 30.67±1.5mEqKg^{-1} (*A. dorsata*). The acidity is due to the minor acid content of honey, mainly aminoacids and organic acids that are responsible for the characteristic taste of honey. The result obtained were statistically insignificant and were in accordance with that of codex alimentarius commission (2001) and were also in line with Attri (2011); Khalil *et al.* (2012) and Selvaraju *et al.* (2019) where pH in the range of 3.62-4.5 has been reported in different honey samples. A highly acidic honey sample indicates the possibility of fermentation of sugars into the organic acid (Ibrahim, 2012; Parihar *et al.*, 2020). Also, according to the obtained results of free acidity, none of the analyzed samples exceeded the limit of 40 mEq Kg^{-1} as required by the international regulations (Bogandov *et al.*, 1999). The significant differences ($p<0.05$) in electrical conductivity was obtained in both the samples. Electrical conductivity is one of the important characteristic of the honey for its authentication which is due to presence of iron and various mineral constituents and organic acid. It was also suggested by Da Silva *et al.*, 2016 that mineral content, organic acids, proteins and acidity influence electrical conductivity of honey. For similar reasons, electrical conductivity of samples of honey of *A. mellifera* and *A. dorsata* showed different electrical conductivity with increased temperature because of weakening of intermolecular forces but results were in accordance to Attri *et al.* (2011) and Baloš *et al.* (2018).

Table 1: Physical properties of honey obtained from *A. mellifera* and *A. dorsata*: pH, Acidity (mEq), Electrical Conductivity (mS^{-1}), moisture (%), Specific gravity (gm^{-1}), Ash content (%), Colour analysis (mAU), Colour range (mAU).

S. No.	Parameter	*A. mellifera*	*A. dorsata*
1	pH	4.16 ± 0.06	4.43 ± 0.21$
2	Acidity	25.67 ± 1.15	30.67 ± 1.5*
3	Electrical Conductivity	1.53 ± 0.1	1.92 ± 0.12#
4	Moisture	18.27±0.12	18.73±0.12*
5	Specific gravity	1.39±0.02	1.4±0.03$
6	Ash content	0.07 ± 0.002	0.57 ± 0.004$
7	Colour analysis	63.43±9.28	125.29±6.89*
8	Colour range	0.27±0.02	0.37±0.02$

Note: Mean ± Standard Error Mean; Mean bearing # -$p<0.05$;* -$p<0.01$; $ -insignificant.

Moisture content of honey affects various other properties like density, specific gravity, refractive index, viscosity and optical properties. Moisture content is a complex function of variables like hygroscopic nature, extraction and handling practices which further depends on time of the year, climatic conditions, the degree of maturation, initial moisture of the nectar, and its geographical origin (Rossant, 2011). If the moisture content exceeds 22 percent honey is likely to ferment (Marvin, 1933). For quick determination of moisture in honey, refractive index method is used. The moisture content of honey was in the reference range of (16%-25%) as mentioned by AOAC (2012) and have significant level of $p<0.01$. Similar studies were reported by Asadi-Dizaji *et al.* (2014); Abdulkhaliq and Swaileh (2017) where moisture content was found to be 16.53% in Palestinian multi-floral honey. The average moisture content of both honey samples was low than those of the Indian AGMARK standard (maximum 22-25%) and international standard (<22) which indicated a proper degree of maturity (Indian Standard Specification; BIS, 1994).

Specific gravity is the ratio of the density of a substance to the density of a reference substance and the value for *A. dorsata* was1.4±0.03 gm^{-1} as compared to *A. mellifera* honey (1.39±0.02 gm^{-1}). The mineral content in honey was generally small and depends on the nectar composition of predominant plants in their formation. The results obtained from analysis of both samples proved to be insignificant and has no significant as far purity of honey was concerned. In present analysis, specific gravity at 30°C ranges from 1.39-1.4 gml^{-1}. A wide range of variation in the specific gravity was reported by many investigations for example, specific gravity ranged from 1.39-1.44 gm^{-1} from Italian honey (Fini, 1966) and 1.30-1.410 gm^{-1} in Japanese honey

(Watanable and Goto, 1956). According to US Standard, standard honey should have specific gravity of 1.406 and the British national Mark Scheme has covered best quality honey if the specific gravity of 1.315 which was in accordance to our study.

The ash content is mainly determined by soil and climatic characteristics and is crucial criterion for appraising the botanical origin of honey. Ash content or mineral content of honey influenced the various characteristics such as colour, taste, flavour, medicinal value, keeping quality and a few physical characteristics (Crane, 1999). In the present analysis, ash content of *A. mellifera* and *A. dorsata* honey samples ranged from 0.07-0.57. However, wide to narrow range (0.03-1.2%) for Indian honey was shown by Das and Bose (1946). Ash content of honey may differ due to difference in floral origin. Dark color honey contains higher ash percentage as compared to lighter honey (Attri, 2011). The ash content of samples investigated in the study was found within acceptable limits i.e. less than 0.6% and was comparable with the findings of Umarani *et al.* (2015).

Data obtained from colour of honey samples revealed that honey sample obtained from *A. mellifera* were of light amber colour and from *A. dorsata* were of dark colour, likewise results were found to be insignificant. Honey color varies naturally in a wide range of tones, ranging from light yellow to amber, dark amber and black (Ibrahim *et al.*, 2012). The determination of color of honey is related to the mineral, pollen and phenolic content of honey (Attri, 2011). Kundal and Kumar (2017) reported that honey of district Kangra have light amber to extra dark amber color which were in cordant to our findings.

Biochemical parameters: Results of analysed honey samples were shown in Table 2.

Table 2: Biochemical properties of honey obtained from *A. mellifera* and *A. dorsata*: Total carbohydrates (gm), Total reducing sugar (g), reducing sugar (g), Fructose (g), Glucose (g), Sucrose (g), Fructose/glucose ratio (%).

S. No.	Parameter	*A. mellifera*	*A. dorsata*
1	**Total carbohydrates**	77.91±0.44	75.84±0.75*
2	**Total reducing sugar**	68.45±0.63	67.28±0.13#
3	**Reducing sugar**	76.54±0.53	74.91±0.52#
4	**Fructose**	35.77±0.74	35.23±0.84$
5	**Glucose**	34.68±0.62	34.45±0.74$
6	**Sucrose**	1.02±0.02	1.02±0.01$
7	**Fructose/glucose ratio**	1.37±0.45	0.93±0.23$

Note: Mean ± Standard Error Mean; Mean bearing # -$p<0.05$;* -$p<0.01$; $ -insignificant.

Carbohydrate analysis of honey is a quality criterion which might influenced by the heating and storage of honey, thus is an indicator of honey freshness. Total carbohydrate content in honey samples were determined by the method of Dubois *et al.* (1956) where total carbohydrates of the examined honey samples were 77.91±0.44 gm for *A. mellifera* and 75.84±0.75 gm for *A. dorsata.* The difference among these values were found to be significant ($p<0.01$). As per Codex Alimentarius Commission (1997), sugar content of honey sample should not be less than 65%. The findings of present study were in accordance with the studies of Khalil (2012) and Biluca *et al.* (2016) where variation in sugar levels were reported (45-70%). The fructose and glucose are the major sugars while sucrose and maltose be the minor ones as honey components. The results of the sugar analysis of honey samples collected from *A. mellifera* showed highest fructose content of 35.77±0.74g/100g as compared to *A. dorsata* honey where it was found to be 35.23±0.84g/100g. The results of the present study were found to be in accordance to the studies of Mahmood and Abbas (2020), where reducing sugars were found in the range of 65.5 to 71.1%. Our results were found to be similar to the studies of Kaushik (1988) where fresh honey samples from Himachal Pradesh were analyzed and 68.33% total reducing sugar levels were observed.

The concentration of fructose and glucose as well as their ratios were considered to be useful indicators for honey quality (Nour, 1988; Oddo and Piro, 2004; Soria *et al.*, 2004 and Buba *et al.*, 2013) as well as its ability to crystallize. Honey crystallization is faster when the Fructose/Glucose ratio is below1.0 (Draiaia *et al.*, 2015). The present study revealed standard value of *A. mellifera* in the ratio of 1.02±0.02 and *A. Dorsata* to be 1.02±0.01 and difference was found to be non-significant. Also, samples were found to be pure in terms of negative Fiehe's and Aniline Chloride Test. Generally, in honey, the glucose is lower than fructose (Siddiqui and Furgula, 1967) and also sugar content contributes only 2-3% of total honey composition. Our results were in concurrent to the observations of many researchers where glucose content in samples obtained from different locations was found to be non-significant. According to Makhloufi *et al.* (2007) the amount of sucrose from Algerian honeys was between 0.1 and 4.3 which was in accordance to the present investigation. The ratio of fructose and glucose affects the crystallization of honey. Results obtained from the present study were in cordant to the studies of Buba *et al.* (2013) where fructose/glucose ratio was found to be in the range of 1.00-1.45.

CONCLUSION

To our knowledge, this is the first time that the physiochemical properties of honey obtained from Nagrota Bagwan bee (*A. mellifera*) were analysed and

compared with the honey obtained from honey bee (*A. dorsata*) of wild origin. The values of the majority of these parameters were widely within the Codex standard for honeys (Alimentarius, 2001) and also considered for freshness index of honey. Both the honey samples has good quality storage capacity and were found to be good for marketing.

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