

Research Article

Bio-chemical analysis of honey made by three, *Apis florea*, *Apis mellifera* and *Apis dorsata*'s honeybee species from Punjab region

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Abstract

A comparative study on the physico-chemical parameters of honey formed via *Apis florea*, *A. mellifera* and *Apis dorsata* honeybees from Punjab region was conducted. Nearly 90 honey samples were gathered from different areas such as Chakwal, Kalar Kahar and Murree. The major flora in these localities were *Acacia*, *Zizipus Jojoba*, *Eucalyptus spp*, Mulberry, Neem, Sheesham and Citrus. Internationally recommended procedure by Honey Commission was undertaken to analyze honey. Water content was away from the maximum limit in almost all samples of honey from *A. dorsata*, whereas, remaining honeys have water % within International limits. Regardless of honeybee species pH was acidic and proline content (above 400mg/kg) measured in all honeys. Lowest average EC (0.10 ± 0.727 mS/cm) and free acidity (12.56 ± 3.226) was found in *A. florea* honey samples comparative to other species. HMF content and Diastases activity in all honeys were within codex limits. However, *A. dorsata* had extremely high invertase number. The existing research established that *A. florea*, *A. mellifera* and *A. dorsata* honeys presented several quality parameters well within International Honey Standards, except water content (exceed the limit of 21%) and *A. dorsata* invertase number may possibly be used as distinguishing factor to identify honey based on honeybee species.

Keywords: *A. dorsata*; *A. florea*; Analysis of honey; Bio-chemical

Introduction

Because of different geographical and floral origin, there are great varieties of honeys

types. Previously several scientists investigated the physical and chemical constituents of honey [1-3]. Generally, honey is valued

through analysis of its physico-chemical constituents as these significant ingredients impact the quality during storage, crystallization, consistency, taste, nutritive and medicinal value of the honey. The configuration of honey largely, subjected towards the plant type exploited by the bees along with area and climatic conditions [4-6]. pH, water% EC, Ash content, various sugar %, minerals, acidity, enzymes activities, HMF content, specific rotation are those components of honey whose determination is set as quality criterion stated by international honey commission (IHC) [7]. As far as honeybee species in Pakistan is concerned, four species inhabited this country. Amongst them *A.cerana*, *A. florea* and *A. dorsata* are native species [8]. However, *A. mellifera*, the fourth species, was brought together in Pakistan during 1976-1977. Commonly *A.dorsata* (called as “doomna”) while *A.florae* (as “choti maki”). Both these honeybee species can't be retained in manufactured modern hives as these are wild in nature and inhabited at open places by making their combs either on trees or buildings. Like other insects, these wild bees also underwent losses mainly in sugar-cane and cotton cultivating areas such as *A.dorsata* colonies decreased to 40,000-50,000 combs. Whereas, only 12,000-15,000 *A.florae* were seen during 1990s. In rural sides honey from *A. florea* is extensively consumed as a normal medication for eye infections, gastro and joint pains, and headache. Current study is planned to find compositional variation in honeys produced by three types of honey bees found across Pakistan.

Materials and Methods

Recommended European Honey Commission and International honey commission procedures were conducted to determine all physicochemical parameters of honeys from three species [11] and values were matched with that of International Honey Standards. About 13% honey solution

in double deionized water was used to measure pH and electrical conductivity [12]. Titration method for 13% honey solution for Free, lactic and total acidities was used [13]. Refractive index and later its correlation with Chataway Charts was used for investigating water % [14]. Similarly, Cough procedure and Winkler's procedure was carried out to determine Proline and HMF content of honey, respectively [15, 16]. Phadebas tablets were used to determine diastase activity [12]. ANOVA [17] was performed for statistical analysis of data. Collection of honey samples from Chakwal, Faisalabad, Bhakkar, Kalar Kahar, shorkot and Murree district of Punjab province was completed.

Results and Discussion

Physicochemical analysis

pH

The results indicate that average pH of *A.florae*, *A.mellifera* and *A.dorsata* is 4.28, 4.66 and 4.45, respectively. Naturally, all honeys pH was acidic. Similarly, pH range start from 3.57 to 6.41. pH is a valuable guide of possible microbial growth. Either neutral or mildly alkaline medium is necessary for bacterial growth, while molds and yeasts require acidic (4.0-4.5) environment for growth and do not propagate thoroughly in alkaline medium [2, 3]. pH values found in honeys of three honeybees species are well with Codex standard and also agreed that are reported previously by Ratiu *et al.* [18]. (4.27) in Romania and Mračević *et al.* [19] (4.30) in Serbia honey. However, less acidic compared to Araujo *et al.* [20] and Adgaba *et al.* [5] (3.8) in Ven-ezuela honey and Ethiopian honey. The acidic pH of honey is needed as acidification is important to stimulate healing due to the release of oxygen from hemoglobin. The average (4.66) *A.dorsata* honey pH existed range was (3.57-5.99), showed notable closeness to (3.4-6.1) reported by Belitz and Grosch [22]. Qamer *et al.* [13] described pH 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) in *A. dorsata* honey. Usually honeys with 5 or above pH, considered as of low pureness and quality [8-10].

Moisture content

A. florea and *A. mellifera* honey samples water content recorded were contained in limits of International Honey Standards, similar to the Water moisture % are reported by Sajid *et al.* [3] (18%), Wu *et al.* [23] (18.10%) and Al-Ghamdi *et al.* [24] (18.5%) in Pakistani, Chinese and Saudi honeys. Though, relative humidity and temperature also upset the water % in the honeys from different geographical origin [25]. Environmental factor, honey crop season and degree of its ripeness also influence honey moisture content [4, 26].

The average (21.7%) and range (19.8-24.6%) of moisture content in *A. dorsata* honey samples was either very close or higher than the prescribed limit by International Honey Standards i.e. 21%. The same greater water % in *A. dorsata* honey is also recorded before by Qamer *et al.* [22] from four (23.5%, 23.99%, 23.91% and 22.2%) different forests of Nepal, [27] (18.9-24.2%), [28] 21%, 27.8% and 23.1%, respectively by [29, 30] from Philippines honey. As this honey bee species forms colonies high in open air so honey possibly diluted due to rain water. Hence homogeneously high water content appears to be the uniqueness of *A. dorsata* honey and may perhaps be used to assess the honey on the basis of honey bee species Qamer *et al.* [22].

Electrical conductivity

EC is measured as a routine quality factor of honey instead of ash content because it's an important standard concerning botanical origin of honey [31]. Its value influenced due to ash and acid content in honey since honey with greater acid content has the higher conductivity. The EC assessed by [23] (0.28mS/cm) in Chinese honey, Sajid *et al.*

[3] (0.26mS/cm) in Pakistani honey, Araujo *et al.* [20] (0.30mS/cm) in Venezuela honey are quite close to the tested samples of *A. mellifera*, although, *A. florea* has low while *A. dorsata* has high EC, nevertheless within customary limit. Qamer *et al.* [22] found 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) in *A. dorsata* honeys from four different forests of Nepal seems close (0.41mS/cm) to the currently found range 0.385-1.3mS/cm.

Acidity

Differences in honey acidity is because of diverse botanical sources along with contrasting honey crop seasons [32]. Free acidity values of all honeys from three honeybee species are lower than the previously stated by [5] (37.1meq/kg in Ethiopian honey and [32] (27.03meq/kg) in Malaysian honey. Qamer *et al.* [22] stated 44.459 meq/kg, 43.169 meq/kg, 43.149 meq/kg and 48.9 meq/kg free acidity in Chitwan district, Central Nepal's *A. dorsata* honey, a little higher than present values. Lactone is also a type of acidity. Same (4.18meq/kg) in *A. florea* and *A. mellifera* and 5.4meq/kg lactone found in *A. dorsata* honey. [20] (9.0meq/kg) in Venezuela honey, [33] (8.79meq/kg) in Chinese honey and [34] estimated lactone (9.5meq/kg) in Portugal honey, higher to present lactone values. Lactone content of *A. dorsata* honey samples were lower to the values 13.2meq/kg, 18.79meq/kg, 15.14meq/kg and 3.5meq/kg described by Qamer *et al.* [22]. [35] noticed the lactone values in Spanish honey at an average of 5.08meq/kg, closer to the present lactone content of *A. dorsata* honey samples. Total acidity is the summation of the free acidity and lactone. It was 16.4meq/kg, 29.48meq/kg and 25.31meq/kg, respectively, in *A. florea*, *A. dorsata* and *A. mellifera* honeys. However, a higher range of acidity has been determined by Sajid *et al.* [3] (46.5meq/kg) in Pakistani honey, [20]

(46.0meq/kg) in Venezuela honey, [20] (35.15meq/kg) in Chinese honey. Likewise, total acidity values of 61.74 meq/kg, 67.98 meq/kg, 60.02 meq/kg, and 56.39 meq/kg has been given by Qamer *et al.* [22] in *A. dorsata* honeys collected from four Terai forests, Nepal. The total acidity range obtained from present study is lower than the reported values.

Proline content

Physiologically all vital essential and non-essential amino acids are naturally part of honey though in minute quantity. Dominant amino acid is proline and its quantity indicate the honey maturity. According to Codex Standard Proline content in normal honeys should be higher than 180 mg/kg. If Proline values are less than 180 mg/kg that means that the honey is possibly adulterated by sugar addition. All the studied honey samples of *A.florea* (402.71mg/kg), *A.dorsata* (239.15mg/kg) and *A.mellifera* (427mg/kg) had proline content above the International recommended limit. On the other hand, lesser proline content (236.21mg/kg and 307.76mg/kg, respectively) were estimated by [33, 36] in Chinese and Malaysian honey. [3, 4] reported 232mg/kg Proline in *A. mellifera* honeys. Qamer *et al.* [22] reported a reduced amount of proline content (98.38mg/kg, 100.8mg/kg, 119.98mg/kg and 160mg/kg in *A. dorsata* honeys collected from Nepal. Some authors establish that honeydew honeys have high proline content. Which also show variation considerably between different honeys [2, 7].

HMF content

HMF is one of the significant constituent to set the freshness and overheating of honey.

Higher HMF value directly expresses about either the inappropriate storage circumstances or honey's presence at higher temperature. In Pakistani *A. florea*, *A. dorsata* and *A. mellifera* honey, average 8.01mg/kg, 27.22mg/kg, 21mg/kg, respectively, HMF content seems lesser as stated by Sajid *et al.* [4] (30mg/kg) in Pakistani honey, [20] (28.9mg/kg) in Spanish honey, [37] (30.10mg/kg) in Amazon honey and [38] (29.67mg/kg) in Malaysian honey previously.

Enzymatic activity

According to White [39] the enzymes in honey are the key proteins and these are added by honeybees themselves through its salvia during the progress of honey maturation. Diastase is the utmost storage and heat resistant enzyme and it should not be less than 8DN in honey as recommended by International Honey Standard. The average enzyme number was 28.77DN in *A. florea*, 23DN in *A. dorsata* and 42.67DN in *A. mellifera*. Sajid *et al.* [4] and Wang *et al.* [40] in Pakistani and Chinese honey also reported diastase well within prescribed level.

Invertase, is another naturally occurring enzyme which regulate the catalysis of sucrose to glucose and fructose and normally used for measuring honey freshness. The invertase activity of 40.29IN and 47.12IN in Tunisia and Bratislava honey determined by [41] and Lichtenberg-Kraag [42] closely related to present invertase activity in *A. mellifera* and *A. florea* honeys (Table 1). However, *A. dorsata* had significantly higher intensity of Invertase number which is also supported by Qamer *et al.* [22].

Table 1: Comparison of physicochemical characteristics of *A. florea*, *A. dorsata* and *A. mellifera* honey

Sr. No.	Parameter	<i>Apis florea</i>		<i>Apis dorsata</i>		<i>Apis mellifera</i>		Codex draft 1999
		Average	Range	Average	Range	Average	Range	
1	pH	4.28±0.798	3.81-6.41	4.66±0.839	3.57-5.99	4.45±0.389	4.31-5.5	
2	Moisture content%	18.27±1.246	15.60-20.20	21.7±1.86	19.8-24.6	18.6±0.91	17.6-21.7	≥21%
3	EC (mS/cm)	0.10±0.727	0.15-0.35	0.41±0.500	0.385-1.3	0.26±1.431	0.28-0.67	≥0.8* mS/cm
4	Free acidity (meq/kg)	12.56±3.226	7.56-18.5	24.16±5.041	9.50-19.0	19±2.051	17-38	≥50 meq/kg
5	Lactone content (meq/kg)	4.18±3.920	2.5-11.75	5.40±4.569	0.5-14	4.17±1.59	3-11	
6	Total acidity (meq/kg)	16.74±4.438	9.50-24	29.48±7.591	4.25-32	25.31±0.839	8-45	
7	Proline content (mg/kg)	402.72±37.36	199.6-577.7	239.15±3.05	142.4-339.1	427±3.67	196-647	≥180* mg/kg
8	HMF content (mg/kg)	8.01±32.104	10.65-20.53	27.22±31.24	19.2-34.30	21±0.77	16-33	≥60mg/kg
9	Diastase number (DN)	28.77±4.731	9.09-23.42	23.00±3.96	9.97-18	42.63±2.09	28-37	≥8
10	Invertase Number (IN)	67.62±32.104	23.84-79.31	390.4±3.04	273-499	39.47±3.65	16-48	≥10

“t test”: 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.

*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.

Conclusion

It can be concluded from the study that although Pakistani honeys produced by different species met the International criteria for good consumable item. Besides its different botanical origin, honey can be distinguishing based on honeybee species keeping its moisture % and Invertase activity.

Authors' contributions

Conceived and planned the research and Did statistical data analysis: S Qamer & M Sajid, Wrote the research paper: Z Haider, QT Awan & B Ahmad, Run the trial and did lab Analysis: S Naz, J Khan, NA Khan & S Sharif.

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