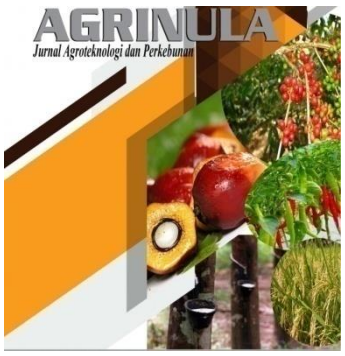

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QUANTIFYING PHYSICOCHEMICAL PROPERTIES OF HONEY COLLECTED FROM DIFFERENT FLORAL SOURCES OF ISLAMABAD AND MARDAN REGIONS

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ABSTRACT

- **Introduction**: Honeybee (*Apis mellifera* L.) is known for pollination and honey production. There are some physicochemical properties through which the honey identifies. The current study was aimed to study the physiochemical characteristics of different honey sources collected from Islamabad and Mardan districts of Pakistan according to the prescribed international standards.
- **Materials and Methods**: This study was conducted in Honey Analysis Laboratory, Honey Bee Research Institute (HBRI), National Agriculture Research Center (NARC) Islamabad, to evaluate the physicochemical properties of honey collected from different floral sources of Islamabad and Mardan regions. The total of 12 honey samples of *A. mellifera* L. were tested. In this study 12 honey samples were collected from beekeepers of Islamabad and Mardan area. Physicochemical analysis such as moisture content, pH, electrical conductivity, free acidity, total soluble solids, density and pollen analysis were carried out.
- **Results**: showed that some of the samples have high

	value for moisture, pH and electrical conductivity while the other parameters were in normal range. Results of the physicochemical properties show the following range of values for moisture content (1.36-1.55%), pH (3.72-6.61), EC (0.1-1.1), acidity (10-30 meq/kg), total sugars (76.2-78.8%), density (1.23-1.46 kg/l), and pollen analysis showed multiple floral samples. **Keywords**: floral sources; honey samples; physicochemical properties

INTRODUCTION

Honey is a delightful sticky sweetener made naturally by bees for their own food from the flower's secretion or nectar (Howes, 2013). It is used as sweeteners and flavoring agents in both alcoholic and non-alcoholic drinks and in confectionaries (Miele et al., 2017). Beekeeping is the practice which is adapted to protect bee colonies (Decourtye et al., 2019) and it is also considered as an important agricultural activity for getting honey and earning a lot of revenue from it (Mohammed et al., 2017). In the genre *Apis*, the majority bees are honey bees and honey bees are known for honey production, pollens, bee venom, wax, propolis and other goods. Besides honey, bees wax, pollen and royal jelly are used in medicines, foodstuffs and other goods (Kampmeier & Irwin, 2009). Honey bees have medical and nutritional benefits and they are also responsible for economic importance for the pollination of agricultural crops (Payne & Van Itterbeeck, 2017). More than 90% of food crops depend heavily on the pollination services of honeybees. There are four species of honeybee which are present in Pakistan, from which two are wild *A. dorsata* and *A. florea* and two are domesticated *A. cerana* and *A. mellifera*. *A. mellifera* L. (Noor et al., 2009). Geographical and environmental factors are the key components which affects quality and composition honey (Pohl et al., 2012). The quality and properties of honey are associated and linked to maturity of honey bee, producing methods, climatic conditions, processing and storage conditions as well as nectar sources of the honey (Guler et al., 2007). Global climate change is disturbing the quality of honey throughout the globe.

Honey is usually evaluated through physicochemical properties of its constituents. Physicochemical parameters like moisture, acidity, pH, hydroxymethylfurfural (HMF) content, color, sugar composition and specific conductivity of the natural honey are precisely defined and each characteristic is known to represent quality indicators and is reported by many scientist (Kahraman et al., 2010; Ndife et al., 2014; Gobessa et al., 2012; Sajid et al., 2020; Kumar et al., 2018). Honey is also well known natural cheaper source of essential inorganic elements for consumers which are required for body metabolism (Al-Waili, 2003). Honey quality has a great impact on texture, flavor, granulation, shelf life and nutritional quality of honey. The current study was aimed to study the physiochemical characteristics of different honey sources collected from Islamabad and Mardan districts of Pakistan according to the prescribed international standards.

MATERIALS AND METHODS

Collection of Honey Samples

A total of twelve honey samples were collected from beekeepers from the different locations of Islamabad and Mardan during the March-April, 2020. Honey samples were stored in sealed plastic jars followed by labeling and dating and kept at the room temperature ± 29°C till completion of analysis. All these honey samples were classified on the basis of their dominant botanical and geographical origin.

Physicochemical Analysis of Honey

The study was completed in the Honey Analysis Laboratory of HBRI, NARC, Islamabad. A total of 12 honey samples of honeybee *A. mellifera* L. were examined in this study. Physiochemical examination of honey samples such as moisture content (%), pH, electrical conductivity (mS/cm), free acidity (meq/kg), total soluble solids (%), density (Baume) and pollen analysis was carried out by the international honey commission (Bogdanov et al., 1999).

Determination of Moisture Content

Moisture of honey must meet international standard (21 g/100g) and is the great cause of honey fermentation throughout the world. The moisture content influences some important characteristics of honey, such as viscosity and brix. Refractometer were used for the purpose of finding the moisture of honey. One drop of honey was taken on the glass surface of refractometer. The sample was covered on the surface of the prism evenly; after two minutes the reading of refractive index was recorded from the lens with the help of eyes.

Determination of pH

pH is another important parameter during extraction and the conservation of honey. It increases the quality, constancy and shelf life of honey (Terrab et al., 2002). pH of 12 honey samples was calculated by pH meter. The pH meter was standardized with standard buffer solution of pH 4 and 7. 10 gram of honey sample was taken and liquefy in 75 ml distilled water in beaker of 250 ml. Solution was stirred and pH electrode was immersed in it, when meter got stable, pH was recorded directly from meter.

Determination of Density

The density was recorded relationship of weight to volume ratio. 1 ml of sample was poured into 5 ml cylinder with the help of a dropper. Weight was measured with laboratory electric balance. Following equation was used for density calculation (Bettelheim & Landsberg, 2012):

$$\text{Density} = \frac{\lfloor(\text{Mass of honey} + \text{cylinder}) - (\text{mass of cylinder})\rfloor}{\text{Volume of honey}}$$

Acidity Determination

Honey (10 g) was weighed with the help of digital analytical balance and dissolved into 75 ml distilled water. Phenolphthalein 1% was used as an indicator. In solution about 4-5 phenolphthalein drops were putted. Then measured beside the solution of 0.1 M NaOH with burette. The titration was carried out till the solution turns to pink from colorless. The acidity was determined by using formula:

Percentage acidity (%)= volume of NaOH used × honey weight

Electrical Conductivity (EC) Calculation

A conductivity meter (Model 315i, WTW instruments Wilhelm, Germany) was used for finding the EC. Distilled water was used to adjust EC Meter after that in 10.0% solution the conducting Cells were putted and when meter got stable EC recorded. 10 grams of honey took in beaker and added 75 ml distilled water. Solution was dissolved well before reading was taken.

Pollen Test

The pollen test of honey was studies according to 10 g of honey was taken in a conical flask. 20 ml of distilled water were added to dissolved the honey in flask. Labeled the sample, then solution was poured in the centrifuge tubes. The centrifuge tubes were then put in the centrifuge in proper balance. The centrifuge was then set at 3000 rpm for 20 minutes. After centrifugation of honey solution, the supernatant liquid was discarded and only the sediment was taken for pollen analysis with the help of droppers. Then the pollen sediments were spread on the slide to check under a compound microscope. The botanical origin of the honey was studied by using the techniques described by Maurizio, (1975). Slides were prepared by acetolysis by centrifuging 10 g of honey dissolved in 20 mL of diluted sulfuric acid (5 g H_2SO_4 L71) for 10 min at 2500 rpm. The supernatant was decanted and the sediment washed twice with 10 mL distilled water and then centrifuged. The sediment was extended on a slide and dried at 70-80 °C, then mounted with stained glycerine gelatin. Pollen grains were identified and counted by using a microscope model BH-2 (Olympus Optical Ltd, Tokyo, Japan) at a magnification of 6200. After pollen grains were counted, they were classified in the following frequency classes: Predominant pollen (445% of the pollen grains counted); secondary pollen (16-45%); important minor pollen (3-15%), and minor pollen (53%), according to Louveaux et al., (1978).

Statistical Analysis

All analysis was carried out in triplicate and the data were expressed as means which was calculated by using Excel (Microsoft Office, version 2013). Standard errors are also calculated and errors bars are assigned to every sample.

RESULTS AND DISCUSSION

Physicochemical Analysis of Honey

The results for physicochemical properties of honey samples collected from Islamabad and Mardan hills are given below in the Figure 1. Figure 1A shows the data for moisture content percentage (MC %), in MC % many factors which includes floral season, degree of maturity and ecological factors determines the moisture contents of honey. Maximum moisture content of honey sample is obtained from Islamabad as 21.9% and it ranges from (19.2-21.9%) while for minimum moisture content is obtained from Mardan as 17% and it ranges from (17-20.9%). According to honey standards set by the Pakistan Standard and Quality Control Authority (PSQCA) and codex, the moisture content of the study area's honey falls under the Grade 'A' category. Density tells us about the glucose contents present in honey at commercial scale. Honey samples with high density have great contents of glucose. In current experiment high density was recorded in Mardan samples and the overall range for density of 6 samples for both regions varies from (1.23-146 kg/l) as show in Figure 1B. Total sugars percentage range from (76.2-78.6%) for samples of both region as show in Figure 1C. pH is an important parameter during extraction and preservation of honey as it raises the shelf life, quality and reliability of honey. pH of different samples obtained from Islamabad and Mardan was given in Figure 1D. Maximum and minimum pH is obtained from honey samples of Mardan as it ranges from (3.72-6.61) while Islamabad honey samples pH range varies from (3.80-5.24). Islamabad honey samples is more acidic in nature as compared to samples of Mardan. pH of honey samples from Islamabad and Mardan lies under the standard category of PSQCA and Codex as given in Table 1. For honey electrical conductivity (EC) depends upon on mineral contents, inorganic ions, organic acids contents and also some sugar complexes and varies with botanical origin. EC is used for detection of quality and purity of honey. For the current sampling of honey EC is shown Figure 1E. Range of EC for honey samples for both regions have a significant difference and it have range from (0.1-1.1) mS cm^{-1}.

Table 1. Physiochemical properties of honey with PSQCA and CODEX Standard

Physiochemical parameter	PSQCA Standard range	Codex standard range
Moisture	Not more than 21%	Not more than 20%
Reducing sugars	Not less than 65%	Not less than 60%
Apparent Sucrose	Not more than 5%	Not more than 5%
pH	3.0-6.40	3.0-6.40
Acidity	Lesser from 40 Meq/kg	Lesser from 50 Meq/kg
Electrical Conductivity	Lesser from 0.8 Ms/cm	Lesser from 0.8 Ms/cm
HMF	Lesser from 80 Mg/kg	Lesser from 40 Mg/kg
Diastase (after blending and processing)	Must not be greater from 3 Shade Units	Must not be greater from 8 Shade Units
Ash	Lesser from 0.6%	Not more than 0.6%
Water insoluble solid contents	Not more than 0.1%	Not more than 0.1%

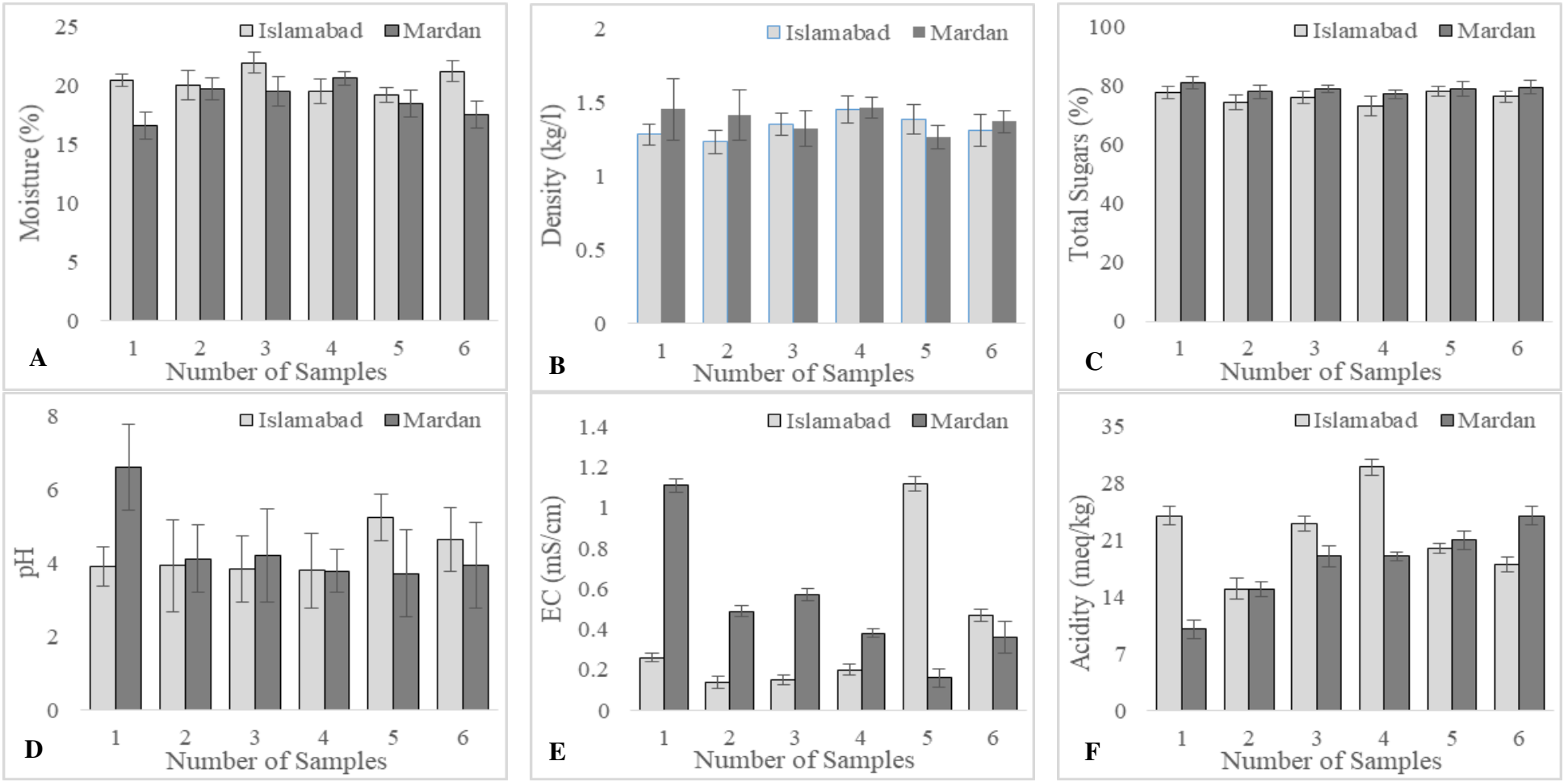

Figure 1. Physiochemical properties of honey bees samples collected from Islamabad and Mardan, A= moisture percentage; B= density percentage; C= total sugars percentage; D= pH; E= electrical conductivity; F= acidity.

There are few samples which are out from the PSQCA and Codex standard from our samples. Due to occurrence of inorganic ions and organic acids, honey is acidic in nature regardless of its origin. Acidity is valuable standard for assessment of honey fermentation and authentication of unifloral honeys. Acidity (meq/kg) for current experimentation is given in Figure 1F, Samples of Islamabad have range of acidity from 15 to 30 meq/kg and Mardan samples have range from 10 to 24 meq/kg. Hence it shows that samples of Islamabad have more organic contents in it and acidic in their physical properties of taste and according to international standards our samples lies in group A for PSQCA and Codex as given in Table 1.

Pollen Test

The pollen of all samples were from medium to high except for one sample S-6 Mardan which showed no pollens. Pollen analysis revealed that some honeys had single flora as primary flora (Uni-floral) such as Sider, Acacia, while most of the samples showed mixed flora. Mardan Sider honey (S-1) sample showed good pollen density.

Table 2. Classification of pollen and pollen density for the studies samples of Islamabad and Mardan

	Pollen		Pollen density	
	Islamabad	Mardan	Islamabad	Mardan
S1	Multi floral	Sider	High	Medium
S2	Multi floral	Multi-flora	Medium	Medium
S3	Multi floral	Acacia + Granda	High	Medium
S4	Brassica, Wild flower	Acacia	Medium	High
S5	Sider	Acacia	Low	High
S6	Accacia, Granda	No pollen	Medium	None

Discussion

Honey composition and quality depends upon various factors such as, weather condition during honey production, composition of nectar, beekeeping practices and handling method during honey extraction and storage. For moisture content percentage our results are supported by the findings of Kumar et al., (2018); Al-Ghamdi et al., (2019) who reported that 18.35-22.1% and 18.50-20.2% water content in different honey samples and these are according to the standards of international organizations. Electrical conductivity (EC) belongs to mobility of electron and is linked with the presence of mineral salt, organic acid and protein levels in honey samples. Kaškonienė et al., (2010) reported that in many regions of "Lithuania" reported EC in floral honeys with range of 0.34 and 0.89 mS cm^{-1}. High mineral contents in honey give us high amount of EC as reported by Alissandrakis et al., (2011). pH and acidity of honey depends upon the presence of organic acids specially gluconic acid as equal to lactones, esters and other chlorides and phosphates (Lanjwani, 2020). Our samples showed presence of organic acids and there range matches and supported by the results of Kinoo et al., (2012). Andrade et al., (1997) reported that composition of honey sugar totally

depends upon with the floral and its region of origin. Our honey samples have different origins and also have multiple floral sources as give in Table 2.

CONCLUSION

The current study revealed that Pakistan, with its highly rich plant flora in hilly areas, is also rich in terms of honey diversity for both Islamabad and Mardan regions. Several types of honey of monofloral origin were compared in this study in addition to multifloral honeys, which investigate the physical and chemical characteristics and the results concluded that honey samples are according with PSQCA and codex standards.

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