**Journal of Animal & Plant Sciences, 33(4): 2023, Page: 991-997**
**ISSN (print): 1018-7081; ISSN (online): 2309-8694**
https://doi.org/10.36899/JAPS.2023.4.0692

# PHYSICOCHEMICAL AND MINERAL CONTENTS OF HONEY FROM DIFFERENT DISTRICTS OF BALOCHISTAN, PAKISTAN

R. Yasmeen[1], F. Behlil[1*], H. Ayub[1], I. Hafeez[2], U. Jabeen[1], S. Rizwan[1], A. Mushtaq[1*], F. Bashir[1] and S. Afzal[1]

[1]Department of Chemistry, Faculty of Basic Sciences, Sardar Bahadur Khan Women's University Quetta, Quetta, Pakistan; [2] PCSIR Laboratories complex Lahore

*Corresponding author's email: farida_behlil@yahoo.com; ayeshamushtaq2000@yahoo.com

## ABSTRACT

Current studies based on the determination of physicochemical properties and mineral content of natural honey samples collected from 14 districts (Barkhan, Chagi, Dera Bugti, Duki, Kachhi, Kharan, Qilla Abdullah, Kohlu, Lasbella, Lorali, Mach, Masky, Sibi and Zhob) of Balochistan, Pakistan. Among physicochemical properties the observed moisture (12.5-7.68%), Ash content (0.50-0.003%), pH (5.49-3.34), total acidity (65-17 meq/kg), Electrical conductivity (0.791-0.150 mS/cm) and colour (ranges dark amber to white). These values indicated that all collected samples meet well with national and international standards. For mineral content Atomic Absorption spectrophotometer is used to detect level of certain metals (Cd, Pb, Cr, Mn, Fe, Cu, Zn, Na, K, Ca, Mg) to detect the pollutions in the districts where sampling was done. However, the honey samples collected from Barkhan districts display best results among all. Principle component analysis (PCA) was applied to differentiate honey samples on the basis of on elemental composition. The results revealed that four groups were formed in which honey samples show correlation with elements.

**Key words:** physiochemical properties, mineral composition, atomic absorption, natural honey, Balochistan.



Published first online April 15, 2023 Published final August 04, 2023

## INTRODUCTION

Honey, a natural sweet substance produces by honeybees that collect the nectar of plants by the process of regurgitation and evaporation which is then stored in honeycomb (Osman *et al*., 2007; Tchoumboue *et al*., 2007). This is one of the most valuable natural substance due to its carbohydrate absorption ability during the period of utilization. It is among the natural and cheaper essential mineral source required by metabolism of body. There are various types of honey depends on types of flora used by honey bee, climate conditions, processing and storage techniques however, the principle constituents in all type are same (Sakac at al., 2019; Sajid *et al*., 2020).

Honey has become traditionally the major commercial natural products in Pakistan (Anjum *et al*., 2015) with four different honeybee species (*Apis cerana, Apis dorsata, Apis florae and Apsi mellifera*) (Qamer *et al*., 2009). *A. mellifera* is the most abundant among these species, which is found to be improve the qualities and quantities of crops by pollination and have an important role in the honey food chain (Sajid *et al*., 2020). Physicochemical parameters such as ash, moisture, pH, acidity, electrical conductivity, colour and mineral contents are known to describe the quality of natural honey. (Waseem *et al*., 2014; Adgada *et al*., 2017; Boussaid *et al*., 2018; Sakac *et al*., 2019) Therefore it is essential to assess the physicochemical and mineral content of various types of honey available in Balochistan and compare these parameters with National and international standards.

The current research is based on the comparative analysis of physicochemical properties and mineral content of honey samples collected from various districts of Balochistan, Pakistan. PCA was applied to differentiate the metal content in honey samples.

## MATERIALS AND METHODS

**Honey samples:** Multifloral type of natural honey samples (n=3) were collected from 14 districts of Balochistan namely Barkhan (S1), Chagi (S2), Dera Bugti (S3), Duki (S4), Kachhi (S5), Kharan (S6), Qilla Abdullah (S7), Kohlu (S8), Lasbella (S9), Lorali (S10), Mach (S11), Masky (S12), Sibi (S13) and Zhob (S14) with the help of local people in the month of April-July 2020. Honey samples were labelled and stored in airtight containers at room temperature and then were transferred to the laboratory at for further analysis.

**Colour analysis:** The honey samples were dissolved in water (1:1 w/v) and the color was measured by means of UV/VIS spectrophotometer at absorbance of 635 nm. The

honey colour was measured by Pfund scale after conversion of the absorbance values: mm Pfund = -38.70 + 371.39 × Abs (Sreckovic *et al*., 2019)

**Physicochemical properties*:*** Various physicochemical parameters such as pH, electrical conductivity, moisture, total acidity and ash contents of the natural honey samples were determined (AOAC, 2002).

The pH was determined by means of a potentiometric pH meter (pH 700 ph/mV/C/F meters, Eutech Instrument) by dissolving 10 g of honey samples in 75 ml of distilled water. Then, the solution was titrated with NaOH (0.1M) to pH 8.3. Free acidity was measured with following equation: Free acidity (meq/kg honey) = mL of 0.1 M NaOH × 10

The electrical conductivity was measured through the electrical resistance. For determination of electrical conductance, honey sample solution (20% w/v) in distilled water was used in conductometer (CON 700 Conductivity/C/F meter, Eutech Instrument).

Moisture content of honey was done by heating the 10 g of honey sample at 100°C in oven until a constant weight was obtained. Ash content was measured by calcinations of 5g honey samples at 550°C in a furnace (VULCAN A-130) to constant weight.

**Biochemical analysis** (Fiehe's test): 5g honey samples were treated with diethyl ether for removal of fat components. Then these samples were treated with freshly prepared resorcinol solution. Production of permanent dark red colour conformed the positive reaction while disappearing of dark colour or conversion into yellow colour indicated the negative reaction (Sharif *et al*., 2018).

**Determination of minerals in honey samples:** For determination of mineral content, 5 g of honey sample in ash form was heated with 5 mL of (0.1 M) nitric acid till dryness. Then 10 mL of (0.1 M) was added to the mixture and volume was made upto 25 mL with deionized water (Silva *et al*., 2009; El-Haskoury *et al*., 2018). The mineral content was determined by atomic absorption spectrometry (Thermo scientific ICE 3000 series AA Spectrometer).

**Statistical analysis:** All the analyses were done in triplicate and results were expresses as mean ± standard deviation. Principal component analysis was performed for analysis of mineral contents on MVSP version 3.22.

## RESULTS AND DISCUSSION

**Physicochemical properties:** All the samples were tested for color, pH, Electrical conductivity, moisture, total acidity and ash content. With the Pfund scale, honey can be classified as water white, extra white, white, extra light amber, light amber, amber and dark amber (Pontis *et al*., 2014; Sreckovic *et al*., 2019). Difference in their color intensity was observed according to the obtained results for analyzed honey samples. According to Vanhanen *et al*., (2011) and Osman *et al*., (2007), dark colour honey samples have higher mineral content such as sulfur, potassium, sodium, magnesium, calcium, copper and iron found in the plant source than the light colour samples. Another factor responsible for the honey's colour is plant species from which the honey bees collect the pollen. Plant species with strong and spicy flavor are found to be responsible for dark colouration while plants with mild and sweet flavor gives light honey colour. The samples S1, S5, S7, S9, S10, and S13 had dark amber colour (245.08±2.76-118.73±5.44 mm Pfund) As these samples sites (Barkhan, Kacchi, Qila Abdullah, Lasbella, Loralia and Sibi) of Balochistan are geographically located as high lands and multiflora plants with strong fragrance are found in these districts (Bibi *et al*., 2015). Sreckovic *et al*., (2019) reported the dark amber (116.07±8.76 mm Pfund) for forest honey samples from Central Serbia. The samples S3, S4, S8 and S11 had light amber colour (79.64±5.51-60.06±1.43 mm Pfund). Compared to honey samples from Spain, which showed higher Pfund value (60±1.00 mm) were classified as light amber colour (Juan-Borras *et al*., 2017) The samples S6 and S12 had extra light amber colour (42.96±2.07-42.58±3.25 mm Pfund). The sample S14 white colour (31.33±0.96 mm Pfund) and S2 had extra white (15.62±2.76) (Table1). Samples sites as Dera Bugti, Duki, Kohlu and Mach are located lower lands while Dalbandin, Masky and Chagi are located in plain areas of Balochistan (Bibi *et al*., 2015) and is the reason of light colour honey. Honey samples from Spain 15 mm Pfund Hungarian had 12±5.00 Pfund and central Serbia 10.04±1.44 Pfund were also categorized as extra white honey (Juan-Borras *et al*., 2017; Sreckovic *et al*., 2019). Some studies have suggested that in honey samples transitional metals can be found react with organic compounds to form a highly colorful complex (Gonzalez-Miret *et al*., 2007; Sreckovic *et al*., 2019). Positive correlation was found among all the analyzed characteristics of the investigated honey samples.

Honey contains different types of acids as aliphatic, aromatic and amino acids. The most common acid reported in honey is gluconic acid produced by the action of glucose oxidase enzyme (Nanda, *et al* 2003; Silva *et al*., 2009; Sreckovic *et al*., 2019; Ratiu *et al*., 2020). Honey samples analyzed were acidic in nature. However, Sample S14 has higher pH value (5.49) followed by S9 (4.70), S10 (4.62), S13 (4.49), S5 (4.46), S3 (4.39), S12 (4.17), S11 (4.02), S1 (3.79), S2 (3.63), S4 (3.58), S7 (3.50), S6 (3.48), S8 (3.34) (Table 2). According to international and national organizations the standard ranges for pH are from 3.0-5.0 for multiflora honey samples (Directive, 2001; Osman *et al*., 2007) and strong fragrance flora of Balochistan (Bibi *et al*., 2015).

Literature agreed with the reported pH values of honey samples (Azeredo *et al*., 2003; Terrab *et al*., 2002; Serrano *et al*., 2004; Sanz *et al*., 2005; El-Haskoury *et al*., 2018). For possible microbial growth pH is a useful index. Neutral and alkaline medium is best for microbial growth, while moulds and yeasts are able to grow in acidic medium (4-4.5) and do not grow in alkaline media (Ratiu *et al*., 2020). In analyzed samples the acidity of the all the samples ranges from 65 to 17 meq/kg. The samples with dark amber colour shown higher acidity as compared with the samples with light amber, very light amber, white, extra white colour showed low acidity Relation of honey colour with acidity was also reported by various researchers (Majewska *et al*., 2019; Ratiu *et al*., 2020).

**Table 1. Colour and Pfund scale values of honey samples from Balochistan.**

| Samples | Colour | Pfund scale (mm) |
|---|---|---|
| S1 (Barkhan) | Dark Amber | 245.08±2.76 |
| S5 (Kachhi) | Dark Amber | 225.87±5.02 |
| S7 (Qilla Abdullah) | Dark Amber | 215.56±8.54 |
| S9 (Lasbella) | Dark Amber | 118.73±5.44 |
| S10 (Lorali) | Dark Amber | 122.21±0.85 |
| S13 (Sibi) | Dark Amber | 142.14±3.15 |
| S6 (Dalbandin) | Extra light amber | 42.58±3.25 |
| S12 (Masky) | Extra light amber | 42.96±2.07 |
| S2 (Chagi) | Extra White | 15.62±0.78 |
| S3 (Dera Bugti) | Light amber | 60.06±1.43 |
| S4 (Duki) | Light amber | 57.75±1.47 |
| S8 (Kohlu) | Light amber | 79.64±5.51 |
| S11(Mach) | Light amber | 79.59±4.56 |
| S14(Zhob) | White | 31.33±0.96 |

**Table 2. Physicochemical parameters of honey samples collected from Balochistan**

| Samples | Moisture (%) | Ash (%) | pH | Acidity (meq/kg) | Electrical Conductivity (mS/cm) |
|---|---|---|---|---|---|
| S1(Barkhan) | 12.5±0.071 | 0.35±0.028 | 3.79±0.01 | 65±0.816 | 0.258±0.002 |
| S2 (Chagi) | 12.45±0.016 | 0.26±0.017 | 3.63±0.01 | 23±1.633 | 0.225±0.002 |
| S3 (Dera Bugti) | 10.88±0.049 | 0.24±0.025 | 4.39±0.02 | 30±2.160 | 0.752±0.002 |
| S4 (Duki) | 10.21±0.024 | 0.05±0.022 | 3.58±0.01 | 21±2.160 | 0.22±0.016 |
| S5 (Kachhi) | 10.18±0.016 | 0.44±0.025 | 4.46±0.02 | 51±2.160 | 0.791±0.001 |
| S6 (Dalbandin) | 10±0.113 | 0.008±0.001 | 3.48±0.01 | 26±2.160 | 0.22±0.022 |
| S7 (Qilla Abdullah) | 9.26±0.037 | 0.011±0.001 | 3.50±0.02 | 42±0.816 | 0.267±0.005 |
| S8 (Kohlu) | 9.01±0.127 | 0.003±0.001 | 3.34±0.01 | 41.4±0.163 | 0.244±0.002 |
| S9 (Lesbella) | 8.96±0.025 | 0.02±0.012 | 4.70±0.01 | 30±2.357 | 0.781±0.001 |
| S10 (Lorali) | 8.75±0.029 | 0.5±0.025 | 4.62±0.02 | 28±0.816 | 0.69±0.028 |
| S11 (Mach) | 8.43±0.008 | 0.05±0.005 | 4.02±0.02 | 23±2.055 | 0.348±0.001 |
| S12 (Masky) | 8.05±0.025 | 0.04±0.009 | 4.17±0.02 | 22±1.633 | 0.15±0.022 |
| S13 (Sibi) | 7.7±0.170 | 0.04±0.016 | 4.49±0.01 | 32±2.449 | 0.65±0.033 |
| S14 (Zhob) | 7.68±0.017 | 0.3±0.141 | 5.49±0.01 | 17±1.414 | 0.711±0.002 |

The electrical conductivity of honey samples is related with concentration of organic acids and mineral salts. Higher organic acid and mineral salts concentration show higher electrical conductivity (Sreckovic *et al*., 2019). Same results were analyzed with higher electrical conductivity was found in sample S5 (0.791 mS/cm) followed by S9, S3, S14, S10, S13, S11, S7, S1, S8, S2, S4, S6 and S12 with electrical conductivity values as 0.781, 0.752, 0.711, 0.690, 0.650, 0.348, 0.267, 0.258, 0.244, 0.225, 0.220, 0.220, 0.150 mS/cm respectively. However, the maximum edible value of electrical conductivity for honey was reported to be 0.8mS/cm in literature (Sreckovic *et al*., 2019; Ratiu *et al*., 2020).

Moisture has important function on those factors which involve in ripening including condition and moisture of nector, condition of storage degree of maturity and harvest season (Terrab *et al*., 2002; Osman *et al*., 2007). Higher moisture content was found in S5 (12.5%) followed by S7 (12.45%), S13 (10.88%), S1 (10.21%), S2 (10.18%), S4 (10%), S9 (9.26%), S3 (9.01%), S14 (8.96%), S6 (8.75%), S8 (8.43%), S12 (8.05%), S10 (7.70%) and S11 (7.68%). The moisture content of all the samples were found to be less than 20%, which was in the ranges as reported in literature. Terrab *et al*., 2002; Osman *et al*., 2007; Kavapurayil *et al*., 2014; Sreckovic *et al*.,2019). Less moisture content in honey is found to be effective for long time storage, as prevents the growth of microorganisms and resist the granulation and fermentation (Chirifie *et al*., 2006).

The ash content is a quality criterion for geographical origin of honey sampling. The ash contents in analyzed honey samples were below the acceptable limits 0.6% (Osman *et al*., 2007). The highest value of ash content was recorded for sample S10 (0.503%), followed by S5 (0.0.44%), S1 (0.35%), S14 (0.30%), S2 (0.26%), S3 (0.24%), S4 (0.048%), S11 (0.046%), S12 (0.039%), S13 (0.038%), S9 (0.020%), S7 (0.011%), S6 (0.010%) and S8 (0.003%). These results suggested the

high pollen source collection by honeybee for production of honey. The physicochemical properties of honey samples found were due the semiarid climatic of Balochistan with annual precipitation varying from 200 to 350 mm. Whereas, evaporation rates are much higher than the precipitation rate (Bibi *et al*., 2015).

All the samples of honey were tested negative for Fiehe's test which confirmed the honey to be free from adulteration (Kavapurayil *et al*., 2014).

**Metal analysis:** The results of the eleven minerals determined in honey samples collected from different districts of Balochistan are shown in Table 3. Three mineral groups can be differentiated: first group composed of elements that are very abundant as K (340-152 mg/kg) followed by Na (85.2-55.2 mg/kg), Ca (62.3-40.27 mg/kg), Mg (19-10.10 mg/kg). The second mineral group in a medium concentration composed of Mn (2.46-1.18 mg/kg), Fe (4.9-2.52 mg/kg), Cu (0.66-4.62 mg/kg) and Zn (0.92-1.93 mg/kg). The third mineral group composed of trace elements as Cd (0.044-0.013 mg/kg) followed by Pb (0.024-0.008 mg/kg) and Cr (0.032-0.01 mg/kg). Magnesium (Mg) was the most abundant element reported the honey samples of Saudi Arabia followed by Fe, Zn, Cu, Mn, Pb and Cd (Osman *et al*., 2007). Similarly, the most abundant elements in the Canary Island were K followed by Na, Ca, Mg Fe, Zn and Cu (Hernandez *et al*., 2005). Whereas, Fe was most abundant element while Cd was reported as lowest element in Turkish honey (Tuzen *et al*., 2007). The dark honeys consist of higher concentration of elements then light honey (Osman *et al*., 2007; Vanhanen *et al*., 2011).

According to the obtained results, K level was the main mineral present in sample from S10 (340 mg/kg) and lowest value was found in S2 (152 mg/kg). The second highest Na level was found in the S5 (85.20 mg/kg) and lowest value was found in S4 (57.2 mg/kg). The reported K and Na levels in all the samples weren't in the health risk to human.

The highest level of Ca was found in the S8 (62.30 mg/kg) and lowest value was found in S2 (40.27mg/kg). The Mg was abundantly reported in S5 (19 mg/kg) and low value was found in S2 (12.71 mg/kg). The presented levels of Ca and Mg in all the samples don't cause health risk to consumers.

The Mn is of low toxicity and biological significant element. The highest level of Mn was reported in the S5 (2.46 mg/kg) and lowest value reported in S2 (1.18 mg/kg). In term of Fe, which is the mostly measured in honey samples, the highest value was reported in the S9 (4.9 mg/kg) and lowest in S2 (2.52 mg/kg). The level of Fe in all the samples was ideal as it was below the guideline value 15 mg/kg (Osman *et al*., 2007) same range with those reported in samples from Turkey (Tuzen and Soylak, 2005), Romania (Oroian *et al*., 2016), and samples from Saudi Arabia (Al-Khalifa and Al-Arify, 1999).

The highest level of Cu content was found in S14 (4.62 mg/kg) and lowest level in S2 (0.66 mg/kg). The highest Zinc values was found in S10 (1.93 mg/kg) and lowest in S11 (0.92 mg/kg) The Cu and Zn concentrations in all the tested samples were found to be below the reported value 5 mg/kg (Osman *et al*., 2007). Both Cu and Zn are essential elements for health but their high intake result in adverse health issues such as gastrointestinal, kidney and liver damage (Al-Khalifa and Al-Arify,1999).

The highest level of Cd was found in S5 (0.044 mg/kg) and lowest in S11 (0.013 mg/kg). All the values of Cd were found to be below the reported level 0.05 mg/kg (Oroian *et al*., 2016). The highest Pb contents were found in S4 (0.002 mg/kg) and lowest in S11 (0.0076 mg/kg). Lead concentration in all the samples was below the reported guideline level 0.1 mg/kg (Osman *et al*., 2007). Same results were reported those in China (Ru *et al*., 2013), and Romania (Oroian *et al*., 2016).

The highest level of Cr was found in S4 (0.032 mg/kg) and lowest in S2 (0.01 mg/kg). The concentration of Cr in all the test samples was below the guideline level 3.70 mg/kg (Waseem *et al*., 2014).

The difference in concentration of mineral contents were due the climatic and flora of different districts of Balochistan (Pirkani *et al*., 2019). All the values of the mineral content was found to be in below then the guideline values as Balochistan is non-industrial region.

**Principal component analysis (PCA) of elemental analysis:** PCA was used for the set of elements to get a strong explainable overview of the main information (Anjum *et al*., 2019). In PCA, Eigen values greater than 1 were separated which result in the formation of three Principal Components (PCs). Highest Eigen value 6.389 was shown by PC1 while PC2 and PC3 resulted as 1.518 and 1.106, respectively. All the three PCs accounted for 81.94% total variance. The maximum variation in data set was shown by PC1 as 58.084%, PC2 as 13.797% and PC3 as 10.056%. Spatial compositional variability in elemental analysis of honey from different district of Balochistan was shown in PCA (Figure-1). PCA variable reported a positive correlation on PC1 with Cd, Pb, Cr, Mn, Fe, Zn, Na, K, Ca and Mg. While a negative correlation was shown with Cu. Similarly, PC2 shown a positive correlation with Cd, Cu, Na, K and Ca. PC3 shown positive correlation with Fe, Cu, Zn, K and Mg. Euclidean bi-plot divided the honey samples into four groups. The first group composed of honey samples collected from district Sibi (S13), Kohlu (S8), Kacchi (S5), Dera Bugti (S3) and Lasbella (S9), being correlated with Na, Ca, K and Cd. The second group composed of Chagi (S2), Masky (S12), Mach (S11) and Kharan (S6)

correlated with no element. The third group composed of Duki (S4), Lorali (S10) and Barkhan (S1) correlated with Cr, Fe, Zn, Mg, Mn and Pb. The fourth group composed of Zhob (S14) and Killi Abdullah (S7) correlated with Cu.

**Table:3 Mineral Composition of Honey from different district of Balochistan.**

| Samples | Cd (ppm) | Pb (ppm) | Cr (ppm) | Mn (ppm) | Fe (ppm) | Cu (ppm) | Zn (ppm) | Na (ppm) | K (ppm) | Ca (ppm) | Mg (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S1(Barkhan) | 0.021 | 0.023 | 0.013 | 1.7 | 3.8 | 1.1 | 1.82 | 67 | 240 | 51.23 | 17.2 |
| S2 (Chagi) | 0.020 | 0.016 | 0.010 | 1.18 | 2.52 | 0.66 | 1.13 | 55.2 | 152 | 40.27 | 10.1 |
| S3 (Dera Bugti) | 0.033 | 0.016 | 0.025 | 2.2 | 4.22 | 1.17 | 1.39 | 81 | 228.2 | 60.3 | 17.13 |
| S4 (Duki) | 0.037 | 0.024 | 0.032 | 2.34 | 4.2 | 1 | 1.37 | 69.37 | 248 | 52.72 | 16.2 |
| S5 (Kachhi) | 0.044 | 0.018 | 0.029 | 2.46 | 4.26 | 1.37 | 1.38 | 85.2 | 325 | 59 | 19 |
| S6 (Dalbandin) | 0.021 | 0.017 | 0.027 | 2.18 | 3.92 | 0.98 | 1.23 | 57.2 | 188 | 48.27 | 17.1 |
| S7 (Qilla Abdullah) | 0.028 | 0.010 | 0.015 | 1.31 | 3.19 | 4.6 | 1.2 | 70.22 | 210 | 52 | 13.95 |
| S8 (Kohlu) | 0.042 | 0.017 | 0.027 | 2.4 | 4.16 | 1.27 | 1.48 | 83.2 | 230.2 | 62.3 | 18.3 |
| S9 (Lesbella) | 0.039 | 0.023 | 0.022 | 2.44 | 4.9 | 3.11 | 1.27 | 69.27 | 328 | 50 | 17.2 |
| S10 (Lorali) | 0.027 | 0.012 | 0.020 | 2.3 | 4.7 | 1.3 | 1.93 | 71 | 340 | 52.7 | 18.82 |
| S11 (Mach) | 0.013 | 0.0076 | 0.012 | 1.73 | 2.78 | 0.67 | 0.92 | 66.2 | 168.2 | 43.27 | 16.37 |
| S12 (Masky) | 0.014 | 0.0086 | 0.012 | 1.66 | 3.01 | 0.87 | 1.12 | 63.12 | 172.2 | 44 | 15 |
| S13 (Sibi) | 0.040 | 0.019 | 0.025 | 2.2 | 4.12 | 1.32 | 1.28 | 84.25 | 235.4 | 61 | 16.2 |
| S14 (Zhob) | 0.027 | 0.010 | 0.012 | 1.28 | 2.99 | 4.62 | 1.23 | 69.33 | 207.32 | 51.32 | 12.71 |

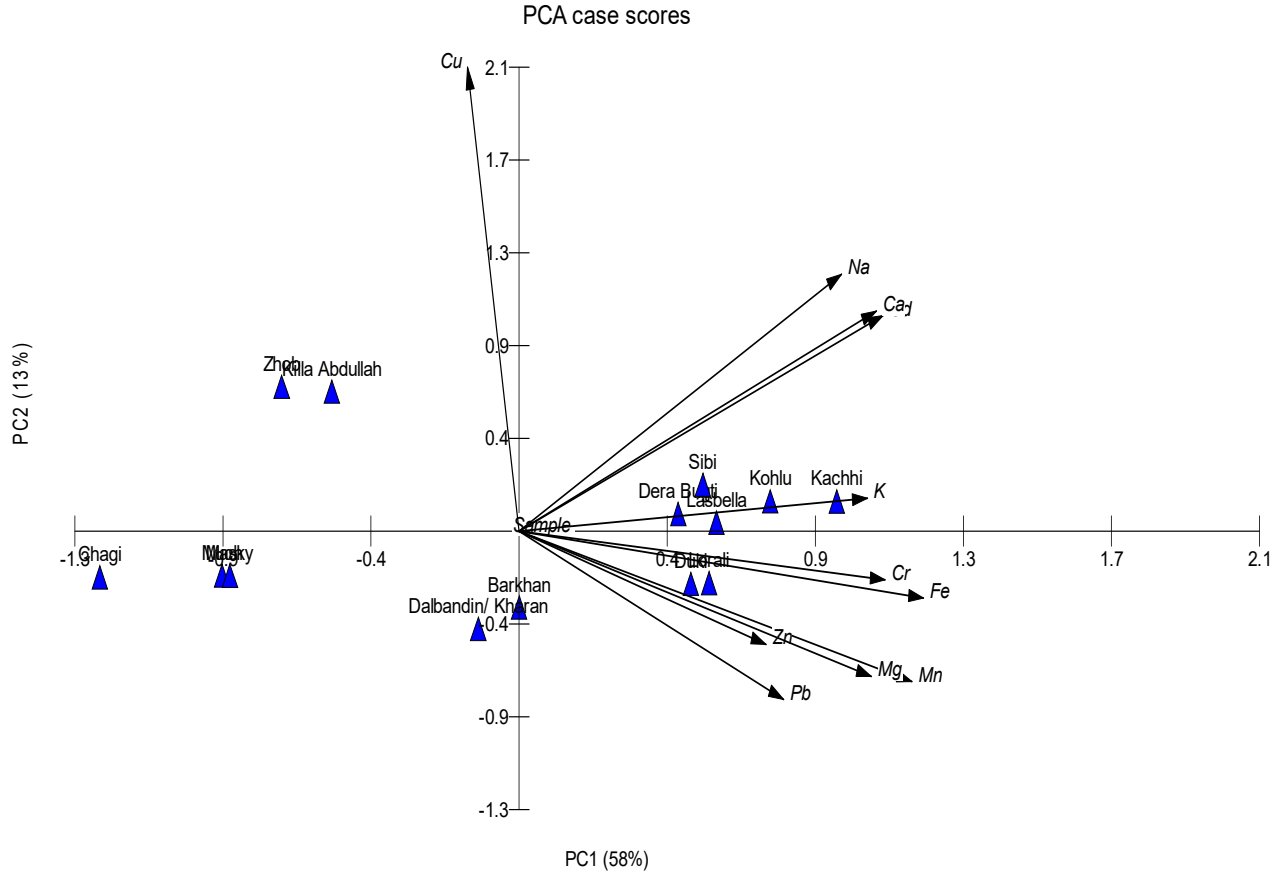


**Figure1: PCA showing Spatial compositional variability in elements in honey samples from different districts of Balochistan.**

**Conclusion:** This study aimed to evaluate and investigate the physiochemical and some metals of natural honey collected from 14 different districts of province Balochistan, Pakistan to confirm their nutritional quality. The results of physiochemical analysis indicates that, all the samples of natural honey meets the national and international standards. The acidic of nature of investigated honey indicates its freshness. The less

moisture contents indicate the resistance of fragmentation in honey. From ash it was concluded that natural source of nectar was flowers. The low concentration of metals in honey samples explains the less polluted - environment of the sampled districts. As these districts were less industrialized. Correlation of metals among the samples were found by PCA. On the basis of Eigen value three principal components were made. Finally the data in the present study concludes that, honey produced in Balochistan does not pose a risk for heavy metals.

## REFERENCES


Adgaba, N., A. A. Al-Ghamdi, A. Getachew, Y. Tadesse, A. Belay, M. J. Ansari, and D. Sharma (2017). Characterization of honeys by their botanical and geographical origins based on physico-chemical properties and chemo-metrics analysis. J. Food Measurement and Characterization, 11(3): 1106-1117. 10.1007/s11694-017-9487-4.

Al-Khalifa, A. S., and I. A. Al-Arify (1999). Physicochemical characteristics and pollen spectrum of some Saudi honeys. *Food chemistry*, *67*(1): 21-25. https://doi.org/10.1016/j.arabjc.2012.11.013

Anjum, S. I., A. H. Shah, M. K. Azim, M. J. Yousuf, S. Khan, and S. N. Khan (2015). Prevalence of American foul brood disease of honeybee in north-west Pakistan. *Biotechnology & Biotechnological Equipment*, *29*(4): 659-66. https://doi.org/10.1080/13102818.2015.1040454

Anjum, S., Z. A. Bazai, S. Rizwan, C. Benincasa, K. Mehmood, N. Siddique and A. Sajjad (2019). Elemental characterization of medicinal plants and soils from Hazarganji Chiltan National Park and nearby unprotected areas of Balochistan, Pakistan. J. Oleo science, 68(5): 443-461. DOI: 10.5650/jos.ess19004

AOAC 2002. Official Methods of Analysis. 17th edn, Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

Azeredo, L., M. A. A. Azeredo, S.R. De Souza and V.M.L. Dutra (2003). Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins. *Food chemistry*, *80*(2): 249-254. https://doi.org/10.1016/S0308-8146(02)00261-3

Bibi, T., M. Ahmad, N. M. Tareen, R. Jabeen, S. Sultana, M. Zafar and S. Zain-ul-Abidin (2015). The endemic medicinal plants of Northern Balochistan, Pakistan and their uses in traditional medicine. J. Ethnopharmacology, 173, 1-10. DOI:10.1016/j.jep.2015.06.050

Boussaid, A., M. Chouaibi, L. Rezig, R. Hellal, F Donsì, G. Ferrari, and S. Hamdi (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. Arabian J. Chemistry, 11(2): 265-274. https://doi.org/10.1016/j.arabjc.2014.08.011

Chirife, J., M. C. Zamora, and A. Motto (2006). The correlation between water activity and% moisture in honey: Fundamental aspects and application to Argentine honeys. J. Food Engineering, 72(3): 287-292.

Directive, C (2001). 110/EC of 20 December 2001 relating to honey. *Official* J. The European Communities, 10(12.1): 2002.

El-Haskoury, R., W. Kriaa, B. Lyoussi and M. Makni (2018). Ceratonia siliqua honeys from Morocco: Physicochemical properties, mineral contents, and antioxidant activities. J. Food and Drug Analysis, 26(1): 67-73. https://doi.org/10.1016/j.jfda.2016.11.016.

Gonzalez-Miret, M. L., F. Ayala, A. Terrab, J. F. Echávarri, A. I. Negueruela and F. J. Heredia (2007). Simplified method for calculating colour of honey by application of the characteristic vector method. *Food research international*, *40*(8): 1080-1086. DOI:10.1016/j.foodres.2007.06.001

Hernandez, O. M., J. M. G. Fraga, A. I. Jiménez, F. Jiménez and J. J. Arias (2005). Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. *Food Chemistry*, *93*(3): 449-458. https://doi.org/10.1016/j.foodchem.2004.10.036

Juan-Borras, M., J. Soto, L. Gil-Sánchez, A. Pascual-Maté, and I. Escriche (2017). Antioxidant activity and physico-chemical parameters for the differentiation of honey using a potentiometric electronic tongue. J. the Science of Food and Agriculture, *97*(7): 2215-2222. DOI: 10.1002/jsfa.8031

Kavapurayil, J., S. Karalam and R. Chandran (2014). Analysis of physicochemical, biochemical, and antibacterial properties of Indian honey samples with special reference to their non-conformity. *Acta Alimentaria*, *43*(1): 9-18. DOI: 10.1556/AAlim.43.2014.1.2

Majewska, E., B. Drużyńska and R. Wołosiak (2019). Determination of the botanical origin of honeybee honeys based on the analysis of their selected physicochemical parameters coupled with chemometric assays. *Food science and biotechnology*, *28*(5): 1307-1314. https://doi.org/10.1007/s10068-019-00598-5

Nanda, V., B.C. Sarkar, H. K. Sharma and A. S. Bawa (2003). Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. J. *Food*

*Composition and Analysis*, *16*(5): 613-619. https://doi.org/10.1016/S0889-1575(03)00062-0

Oroian, M., A. Prisacaru, E. C. Hretcanu, S. G. Stroe, A. Leahu and A. Buculei (2016). Heavy metals profile in honey as a potential indicator of botanical and geographical origin. *International* J. Food Properties, 19(8): 1825-1836. https://doi.org/10.1080/10942912.2015.1107578

Osman, K. A., M. A. Al-Doghairi, S. Al-Rehiayani and M.I. Helal (2007). Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia. J. Food Agriculture and Environment, *5*(3/4): 142.

Pirkani, Z. A., M. Asrar, S.K. Leghari, M. A. Dashti, and M. Manzoor (2019). Nutritional and mineral content of three wild agaricus species of district Mastung Balochistan, Pakistan. *FUUAST* J. Biology, *9*(2): 227-231.

Pontis, J. A., L. A. M. A. D. Costa, S. J. R. D. Silva and A. Flach (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology*, *34*(1): 69-73. https://doi.org/10.1590/S0101-20612014005000015

Qamer, S., N. Muzaffar, S. S. Ali, and A. R. Shakoori (2009). Effect of storage on various honey quality parameters of unifloral sidder honey from Pakistan. Pakistan J. Zoology, *41*(4).

Ratiu, I. A., H. Al-Suod, M. Bukowska, M. Ligor and B. Buszewski (2020). Correlation study of honey regarding their physicochemical properties and sugars and cyclitols content. *Molecules*, *25*(1): 34. https://doi.org/10.3390/molecules25010034

Ru, Q. M., Q. Feng and J.Z. He (2013). Risk assessment of heavy metals in honey consumed in Zhejiang province, southeastern China. *Food and chemical toxicology*, *53*: 256-262. DOI: 10.1016/j.fct.2012.12.015

Sanz, M. L., M. Gonzalez, C. De Lorenzo, J. Sanz and I. Martınez-Castro (2005). A contribution to the differentiation between nectar honey and honeydew honey. *Food chemistry*, *91*(2): 313-317. doi:10.3390/agriculture2010025

Sajid, Z. N., M. A. Aziz, I. Bodlah, R. M. Rana, H. A. Ghramh, and K. A. Khan (2020). Efficacy assessment of soft and hard acaricides against Varroa destructor mite infesting honey bee (Apis mellifera) colonies, through sugar roll method. *Saudi* J. Biological Sciences, *27*(1): 53-59. doi.org/10.1016/j.sjbs.2019.04.017

Serrano, S., M. Villarejo, R. Espejo and M. Jodral (2004). Chemical and physical parameters of Andalusian honey: classification of Citrus and Eucalyptus honeys by discriminant analysis. *Food Chemistry*, *87*(4): 619-625. DOI;10.1016/j.foodchem.2004.01.031

Sharif, A., M. Iftikhar, A. Hussain, M. U. Rehman, S. F. Zaidi, M. Akram and K. Usmanghani (2018). Evaluation of Physio-chemical properties of honey collected from local markets of Lahore, Pakistan. *Pak J Med Biol Sci*, *2*(1).

Sakac, M. B., P. T. Jovanov, A. Z. Maric, L. L. Pezo, Z. S. Kevresan, A. R. Novakovic, and N. M. Nedeljkovic (2019). Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia). *Food chemistry*, *276*, 15. DOI: 10.1016/j.foodchem.2018.09.149

Silva, L. R., R. Videira, A. P. Monteiro, P. Valentão and P. B. Andrade (2009). Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemical* J. *93*(1): 73-77. https://doi.org/10.1016/j.microc.2009.05.005

Sreckovic, N. Z., V. B. Mihailovic and J. S. Katanic-Stankovic (2019). Physico-chemical, antioxidant and antimicrobial properties of three different types of honey from Central Serbia. Kragujevac J. Science, (41): 53-68. https://doi.org/10.5937/KgJSci1941053S

Tchoumboue, J., J. Awah-Ndukum, F. A. Fonteh, N. D. Dongock, J.M.Z.A. Pinta and Z. Mvondo (2007). Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon. African J. Biotechnology, 6(7)

Terrab, A., M. J. Dıez and F. J. Heredia (2002). Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food chemistry*, *79*(3): 373-379. https://doi.org/10.1016/S0308-8146(02)00189-9

Tuzen, M., S. I. B. E. L. Silici, D. Mendil, and M. Soylak (2007). Trace element levels in honeys from different regions of Turkey. *Food chemistry*, *103*(2): 325-330. https://doi.org/10.1016/j.foodchem.2006.07.053

Tuzen, M., M. Soylak (2005) Trace Heavy Metal Levels in Microwave Digested Honey Samples from Middle Anatolia, Turkey. J. Food and Drug Analysis, 13: 343–347. https://doi.org/10.38212/2224-6614.2571

Waseem, A., J. Arshad, F. Iqbal, A. Sajjad, Z. Mehmood and G. Murtaza (2014). Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. *BioMed research international*, Article ID 813206, 29 pages, 2014. https://doi.org/10.1155/2014/813206

Vanhanen, L. P., A. Emmertz, and G. P. Savage (2011). Mineral analysis of mono-floral New Zealand honey. Food Chemistry, *128*(1): 236-240. DOI: 10.1016/j.foodchem.2011.02.064.