

## Quality Analysis and Elemental Presence of Heavy Metals in Honey

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### Abstract

Honey is a naturally occurring material made by honeybees from the sweet, savory nectar of flowers. Humans have consumed honey for a very long time, making it the oldest sweetener and health food. Honey was mentioned in Egyptian, Indian, and Chinese texts as early as 5500 BC. It is renowned for its therapeutic and nutritional properties brought on by beneficial elements that are strongly connected to its flower origin. Although the basic variation in honey composition is due to the floral source, other elements such as the season, the environment, and the processing conditions also have a role. The sensory, chemical, physical, and microbiological properties of honey are mostly linked to its quality. Honey should indicate the minimum quality standards to be marketed as honey or employed as an ingredient in products for human consumption. Physico-chemical characteristics including pH, water content, ash content, HMF and EC parameters were analyzed which showed that Pakistani honey is under the limits. Additionally, the limited presence of heavy metals was also seen within the range. Pakistani honey is one of the best honeys to be used for raw consumption as well as raw material for any value-added products.

**Keywords:** Quality analysis; honey in Pakistan; physico-chemical characteristics of honey; heavy metal presence.

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## INTRODUCTION

Honey is a naturally occurring material made by honeybees from the sweet, savory nectar of flowers. Honey is a really unique, healthful, and beneficial food. Since the beginning, it has been utilized as a food and medicine (Tafere, 2021). It is renowned for its therapeutic and nutritional properties brought on by beneficial elements that are strongly connected to its flower origin (Gündoğdu *et al.*, 2019). Additionally, the nectar, which is used to make honey, and the conditions of handling and storing it all affect the composition and quality of the honey (Jain, 2021). This organic substance is an aqueous concentrated solution of various carbohydrates, including fructose, glucose, maltose, sucrose, and other oligo- and polysaccharides (Amessis-Ouchemoukh *et al.*, 2021). Also It includes certain trace amounts of proteins, enzymes, amino and organic acids, lipids, vitamins, minerals, flavorings, flavonoids, and phenolic compounds (Mohammed, 2022; Pavlova *et al.*, 2018). However, due to various chemical reactions like

fermentation, oxidation, and heat processing, honey components might alter while being stored (Singh & Singh, 2018).

The proportion of honey depends primarily on the floral origin, but also on the season, location, and processing conditions (Tafere, 2021). Simple carbs, including fructose (38.5%) and glucose (31%) make up 82.4% of the total carbohydrates in honey. Maltose, sucrose, and other sugars make up the remaining 12.9% of the carbs (Lestari & Lestari, 2019). Depending on the species of honeybees, honey has a different protein composition. Protein, for instance, fluctuates between 0.2% and 1.6% in *Apis mellifera*, whereas it varies between 0.1% and 3.3% in *Apis cerana*. Honey contains 1% amino acid content. Although proline, which makes up 50–85% of all amino acids, is the most prevalent (Gündoğdu *et al.*, 2019). The worker honeybees' hypopharyngeal glands are the source of the enzyme amylase, glucose oxidase, and invertase; however, the other three enzymes in honey come from different

places. On the other side, plants are the source of catalase, acid phosphatase, and a very little quantity of amylase. Honey has a very low mineral and vitamin content, making only just 0.02% of its weight (Tarapatsky *et al.*, 2021). The 0.57% organic acids in honey are what give it its acidity. Alternatively, some inorganic acids lead to free acidity, which is crucial for honey degradation. Storage affects acid content, lactic acidity, invertase activity, and hydroxymethylfurfural (Brugnerotto *et al.*, 2021).

Due to its long-standing medicinal and culinary benefits, honey is a common component in traditional medicine (Dai *et al.*, 2021). The floral source of the honey greatly influences its functional capabilities in human health, which may be connected to honey's high osmolarity and antimicrobial properties (Hossain *et al.*, 2022). Polyfloral honeys have stronger antioxidant activity than monofloral honeys because they include more flavonoid and phenolic components. Fructose, glucose, and water make up the majority of the more than 200 ingredients in honey (Sultana *et al.*, 2020).

Honey is produced in Pakistan on a huge scale, and the country is currently ranked 20th in the world for its production of raw honey, with yearly output of more than 15,000 metric tons. Since a few years ago, honey has emerged as one of the most commercial agricultural products available for trade in Pakistan. The primary goals of this research study were to observe the physicochemical properties of honey produced in Pakistan and to develop the baseline information.

## MATERIALS AND METHODS

### SAMPLES PREPARATION

The honey samples (15 in numbers) were collected from randomly selected areas of Pakistan. The samples were collected in bottles/jars. Samples were properly labeled with numbers, location names, and the date of collection. Before testing these samples,

undesirable materials such as dead bees, wax sticks, and comb particles were also removed from the honey. All honey samples were kept at the IFNS lab facility before analysis. The analyses were conducted in the labs facilities of IFNS and lab of department of environmental sciences (FCFS) for ICP analysis.

### PHYSICO-CHEMICAL CHARACTERISTICS

The pH of honey was determined using pH meter by the method of Bogdanov *et al.*, (2002). The amount of water in honey samples was calculated by refractive index using the method of Bogdanov *et al.*, (2002). The ash content was evaluated using the method defined by Bogdanov *et al.*, (2002). EC was determined using EC meter by the reference method (Bogdanov *et al.*, 2002). The elemental analysis was performed by ICP utilizing the reference method of Yayinie & Atlabachew (2022).

### STATISTICAL ANALYSIS

The physicochemical parameters of honeys were analyzed, and the results were presented as means. Bar graphs were created in order to facilitate the analysis and examination of data. Analysis of variance (ANOVA) was employed to assess the physiochemical parameters, such as pH, moisture, ash content, HMF, EC and heavy metals under suitable settings. The results were statically analyzed using the collected data (Steel *et al.*, 1997).

## RESULTS AND DISCUSSIONS

Fifteen honey samples were gathered from randomly selected regions in Pakistan and subsequently subjected to analysis. The mean of all samples was compared using column graphs.

### PHYSICO-CHEMICAL CHARACTERISTICS

The physicochemical qualities of honey sourced from Pakistan were assessed, and subsequent honey-based products were formulated.

**Table 1: Mean values of physiochemical characteristics**

Honey Samples	pH	Moisture (%)	Ash (g/100g)	HMF (mg/kg)	EC (mS/cm)
H1	3.32	15.66	0.15	33.50	0.33
H2	3.43	15.95	0.17	37.42	0.37
H3	4.30	17.69	0.17	30.50	0.36
H4	4.06	18.86	0.16	22.59	0.36
H5	4.58	19.30	0.14	19.80	0.34
H6	3.73	17.41	0.17	17.53	0.28
H7	3.70	17.39	0.13	40.00	0.23
H8	4.89	18.51	0.12	28.83	0.68
H9	3.38	17.30	0.15	35.60	0.50
H10	4.84	16.97	0.19	36.00	0.47
H11	4.60	19.43	0.34	33.35	0.41
H12	4.59	18.21	0.18	31.05	0.45
H13	5.12	20.14	0.15	21.50	0.25
H14	3.91	17.31	0.13	41.35	0.43
H15	3.87	18.05	0.14	34.85	0.60

## pH

As shown in Table 1, the pH values of natural honeys gathered from different locations of Pakistan had a relationship to one another. The pH values of these honeys fell within the range of 3.32 to 5.12 in this research study. The acidic properties exhibited by honey can be attributed to the presence of certain metal ions, phenolics, amino acids, and gluconic acid. The acidity exhibited by honeys from Pakistan not only indicates

their remarkable antibacterial properties, but also serves as a beneficial therapeutic agent due to their ability to facilitate the quick release of oxygen from hemoglobin. The sample with the lowest amount of pH was found to be sample one with 3.32, and the sample with the highest amount of pH was sample thirteen with 5.12. The values of pH of honey found in this research are like the findings of Mehryar *et al.*, (2013).

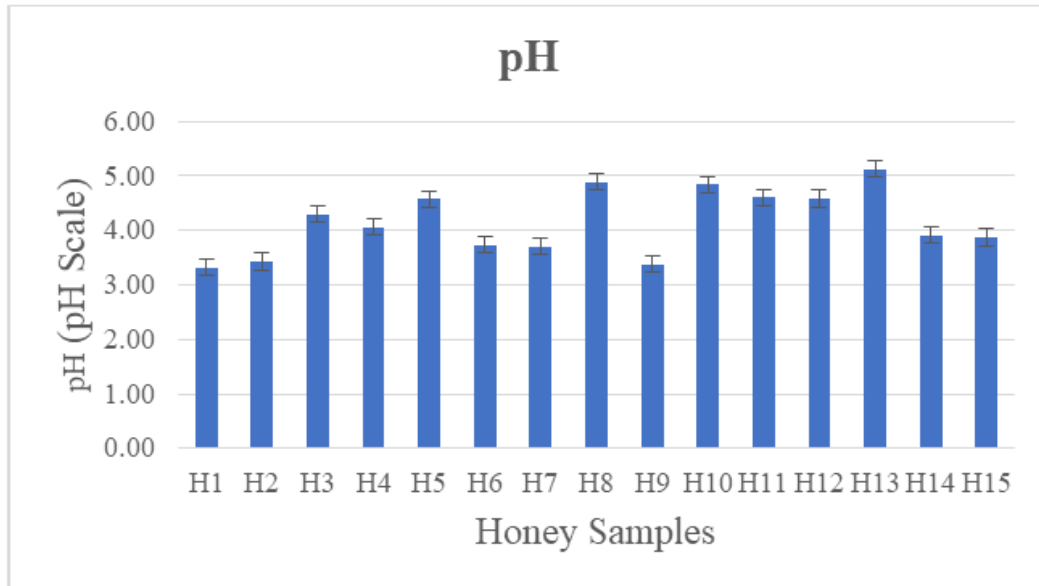


Figure 1: Bar graph for pH of honey samples

## Water Content

The amount of moisture that is contained within the honey is a highly essential metric, since it contributes to the honey's stability against granulation and fermentation during storage (Kumar *et al.*, 2018). The

most measurements placed the level of moisture content somewhere in the range of 20.14% to 15.66% as shown in Table 1. The values of moisture contents of honey found in this research are similar to the findings of Iftikhar *et al.*, (2014).

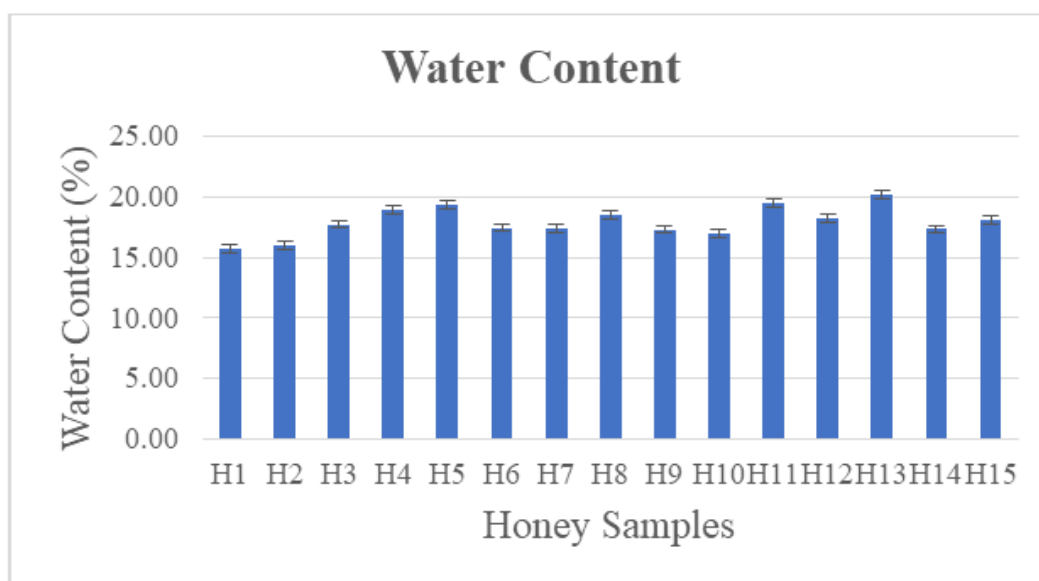


Figure 2: Bar graph for water content of honey samples

### Ash Content

One such factor used to identify the plant source is the amount of ash present. According to Bouhlali *et al.* (2019), ash is a direct assessment of the inorganic residues left over after honey is carbonized. Table 1 displays the results of an analysis of the ash content of various honey samples, which ranged from 0.12 to 0.34

g/100gm. The findings are like the study of Ahmed *et al.*, (2016). The sample with the lowest amount of ash content was found to be sample eight with 0.12 g/100g, and the sample with the highest amount of ash content was sample eleven with 0.34g/100g as shown in Figure 3.

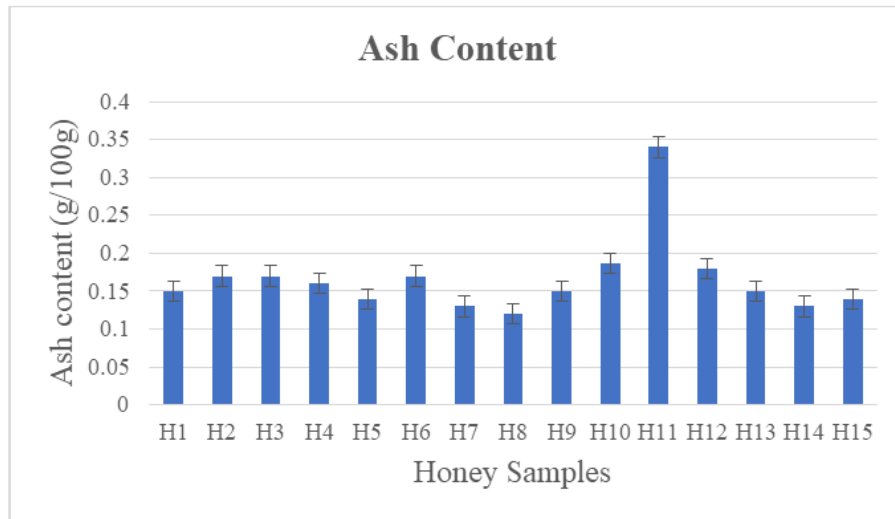


Figure 3: Bar graph for ash of honey samples

### Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is a derivative compound resulting from the degradation of fructose, a prominent saccharide found in honey. Its formation occurs gradually and spontaneously during the storage of honey, whereas elevated temperatures expedite its production. The application of high heat treatment during honey processing or extended storage at temperatures exceeding 27°C leads to an elevation in the levels of hydroxymethylfurfural (HMF). It is recommended that the content of Hydroxymethylfurfural (HMF) does not

surpass 40 mg/kg. The HMF content observed in the present investigation exhibited a range of 17.53 to 41.35 mg/kg, as depicted in Table 1.

Boussaid *et al.*, (2018) reported similar HMF content of honey as reported in the present research. The sample with the highest percentage of total sugar was found to be sample nine with 65.44%, and the sample with the lowest percentage of total was sample six with 86.60% as shown in Figure 4.

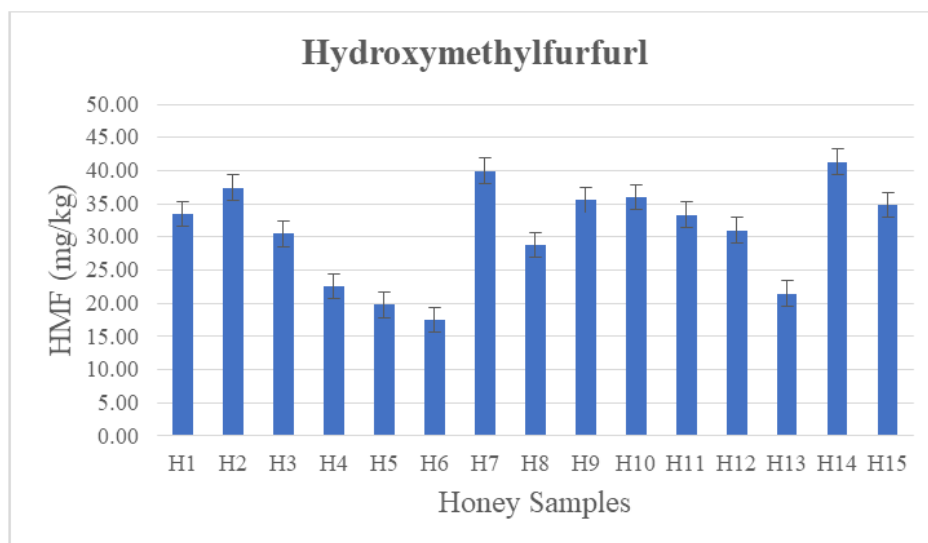


Figure 4: Bar graph for HMF of honey samples

**Electrical Conductivity**

Honey from the same geographical origin can have significantly different EC values depending on the species of honeybee that produced it. The mineral make-up of honey is directly related to the amount of electrical conductivity it possesses. Honey's electrical conductivity can be a useful indicator of its botanical source. Electrical conductivity values of honey samples were found to range from 0.23 to 0.68 mS/cm, as shown in Table 4.8.

The sample with the highest amount of EC value was found to be sample eight with 0.68 mS/cm, and the sample with the lowest amount of EC value was sample seven with 0.23 mS/cm as shown in Figure 5. Nevertheless, the electrical conductivity (EC) values of all the Pakistani honeys that were examined were found to be within the acceptable thresholds set by international honey standards (Iftikhar *et al.*, 2014).

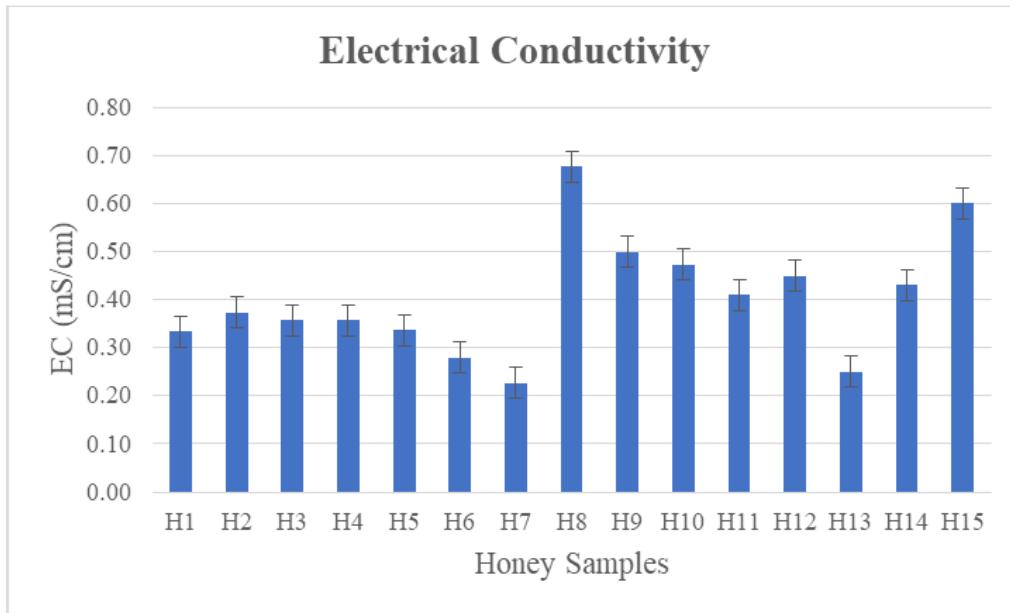


Figure 5: Bar graph for EC of honey samples

**4.2 MULTI-ELEMENTS ANALYSIS**

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have collaboratively issued a recommendation for the Provisional Tolerable Weekly Intake (PTWI) of hazardous substances that can be consumed without

posing significant health risks on a weekly basis. The purpose of this study was to investigate the presence of hazardous metals that have the potential to accumulate in various organs of the body following the process of digestion (Adugna *et al.*, 2020).

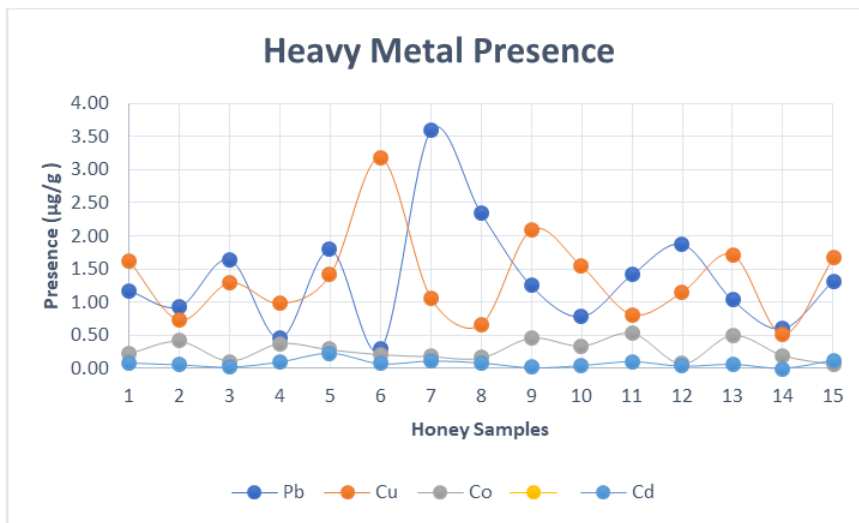


Figure 6: Mean values of presence of heavy metals in honey samples

**Table 3: Mean values of presence of heavy metals in honey samples**

Honey Samples	Pb	Cu	Co	Cd
H1	1.17	1.62	0.22	0.08
H2	0.93	0.73	0.41	0.05
H3	1.64	1.29	0.11	0.02
H4	0.45	0.98	0.37	0.10
H5	1.79	1.41	0.28	0.23
H6	0.28	3.18	0.21	0.07
H7	3.58	1.06	0.18	0.11
H8	2.34	0.66	0.16	0.08
H9	1.24	2.09	0.46	0.01
H10	0.78	1.54	0.34	0.04
H11	1.41	0.81	0.53	0.10
H12	1.87	1.15	0.07	0.03
H13	1.03	1.72	0.50	0.06
H14	0.60	0.52	0.19	0.00
H15	1.32	1.66	0.06	0.12

**Copper (Cu)**

Table 2 presents the copper concentrations of the honeys examined, which vary between 0.52  $\mu\text{g/g}$  and 3.18  $\mu\text{g/g}$ . The concentration found is similar to the findings of Silva *et al.*, (2021). Crop mineralization, environmental contamination, and the use of herbicides, pesticides, fungicides, and bactericides in the surrounding area contribute to the presence of copper in food products (Džugan *et al.*, 2017).

**Lead (Pb)**

The lead concentration in honey samples from Pakistan was below the permissible limit. Lead contents of analyzed Pakistani honeys ranges from 0.28  $\mu\text{g/g}$  to 3.58  $\mu\text{g/g}$  are given in the Table 2. The concentration found is similar to the findings of Silva *et al.*, (2021). The sample with the highest value of Pb presence was found to be sample seven with 3.58  $\mu\text{g/g}$  and the sample with lowest value Pb was sample six with 0.28  $\mu\text{g/g}$  as shown in Figure 1.

**Cobalt (Co)**

Cobalt contents of analyzed Pakistani honeys ranges from 0.06  $\mu\text{g/g}$  to 0.53  $\mu\text{g/g}$  are given in the Table. The sample with the highest value of cobalt presence was found to be sample eleven with 0.53  $\mu\text{g/g}$  and the sample with lowest value cobalt was sample fifteen with 0.06  $\mu\text{g/g}$  as shown in Figure 1. The concentration found is similar to the findings of Silva *et al.*, (2021).

**Cadmium (Cd)**

The concentrations of Cd in the Pakistani honeys analyzed range from 0.02  $\text{g/g}$  to 0.23  $\text{g/g}$ , as shown in Table 2. One of the fifteen samples did not contain Cd. The presence of high concentrations of Cd in numerous Pakistani honeys indicated the toxicity of Cd in these honeys. The concentration found is similar to the findings of Silva *et al.*, (2021). The sample with the highest value of Cd presence was found to be sample five with 0.23  $\mu\text{g/g}$  and the sample with lowest value cobalt

was sample three with 0.02  $\mu\text{g/g}$  while sample fourteen didn't find any contamination of Cd as shown in Figure 2.

**CONCLUSION**

Honey is an organic compound that is synthesized by honeybees through the enzymatic conversion of floral nectar, which contains both sweet and savory components. Honey is a special food that is also very useful to your health. The physiochemical analysis was done including pH, water content, ash content, HMF and EC. The results for these parameters have significant values.

The pH values of these honeys fell within the range of 3.32 to 5.12. The most measurements placed the level of moisture content somewhere in the range of 20.14% to 15.66%. The ash content was found ranges from 0.12  $\text{g}/100\text{g}$  to 0.34 $\text{g}/100\text{g}$ . The HMF content observed in the present investigation exhibited a range of 17.53 to 41.35  $\text{mg}/\text{kg}$ . Electrical conductivity values of samples were in range from 0.23 to 0.68  $\text{mS}/\text{cm}$ .

The copper concentrations vary between 0.52  $\mu\text{g/g}$  and 3.18  $\mu\text{g/g}$ . Lead contents of samples ranges from 0.28  $\mu\text{g/g}$  to 3.58  $\mu\text{g/g}$ . The cobalt presence was found from 0.53  $\mu\text{g/g}$  to 0.06  $\mu\text{g/g}$ . The concentrations of Cd in the Pakistani honeys range from 0.02  $\text{g/g}$  to 0.23  $\text{g/g}$ . One of the fifteen samples did not contain Cd. All were within the limits set by International Honey Commission.

This study suggests developing value added products from Pakistani honey to boost the country economy. The honey from Pakistan has the potential quality to design further research studies with different goals to explore in other ways.

**Conflict of Interest:** There is no conflict of interest among the authors.



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