



Physico-Chemical Properties and Determination of Heavy Metal Levels in Honey by Atomic Absorption Spectroscopy

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ABSTRACT

Physicochemical properties and heavy metals in honey are of interest not only for quality control but also for determination environmental contamination. The present study was carried out to investigate the physicochemical properties and heavy metal levels in branded and non branded honey samples purchased from different areas of Peshawar, Islamabad, Mardan, Swabi and Charsadda. The main objective of this study was to compare the branded and non branded honey samples obtained from the different regions. The collected honey samples were analyzed for pH, ash content, moisture and electrical conductivity and four heavy metals i.e. Iron, Lead, Copper & Zinc and levels of these metals were determined using atomic absorption spectroscopy. The samples in this study were prepared by ashing method. The concentration ranges obtained for the metals analyzed in honey samples were as follows; Fe (1.26 ± 0.05 - 7.21 ± 2.01), Pb (0.07 ± 0.06 - 0.53 ± 0.03), Cu (0.02 ± 0.01 - 0.17 ± 0.01) and Zn (0.81 ± 0.13 - 7.73 ± 0.02) in mg/kg. The results shows that the geological strata may affect the physicochemical properties and the metal content in the honey samples.

Keywords: Honey samples, physicochemical properties, heavy metals, AAS.

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INTRODUCTION

Heavy metal can be defined as “Those metals which have density more than 5g/cm^3 , atomic weight 63.546 to 200.590 and a specific gravity greater than 4.0”^{1,2}. Honey formed an important dietary and medical component of civilizations across the globe including Egypt and the Middle East, China, Mesoamerica (Mayan) and Rome. This role has continued throughout the ages into modern times³. Honey also contains numerous types of sugars, as well as acids, proteins and minerals⁴. Many scientists reported regional variation in the physicochemical properties of the honey samples, such as the ash contents, enzymes activities, hydroxymethylfurfural (HMF), electrical conductivity, pH and specially their metal content⁵. When honey is dried and burned, a small residue of ash remains, which is the mineral content⁶. Honey contains varying amounts of mineral substances ranging from 0.02 to 1.03g/100g. Honeydew honey is richer in minerals and due to its mineral content is said to be less suitable for storage in the winter. Several investigations have shown that the trace element content of honey depends mainly on the botanical origin of honey, light blossom honeys having a lower content than dark honeys, e.g. honey dew, chest nut etc. In early times the mineral content was determined as a quality criterion of honey⁷. The main trace elements found in honey are Mg, Ca, Al, Fe, Mn, Zn, B, Cu, Co, Cr, Ni, Cd and P⁸. When honey is dried and burned, a small residue of ash remains, which is the mineral content⁶. Honey contains varying amounts of mineral substances ranging from 0.02 to 1.03g/100g. Honey dew honey is richer in minerals and due to its mineral content is said to be less suitable for storage in the winter. The nectar, from which the honey is made, contains metals absorbed by the roots from the polluted soil, and may also contain metals carried by the bees from polluted water sources^{6,9}. High concentration of metals in honey can be a source of illness to human beings, especially heavy metals which are related to health problems in man. It has been reported that lead can cause damage of brain, kidney, nervous system and red blood cells. Other problems caused by heavy metals include metabolic anomalies, respiratory disorders, nausea and vomiting. Poor methods of honey harvesting, processing and storage are another source of change in the mineral content in honey^{10,11}.

The most abundant minerals found in Pakistani honey samples were K, Na, Ca, Mg, Fe, Zn, Cu, Ni and Co. The levels of Fe, Cu, Zn, Pb, Ni and Mn were determined using atomic absorption spectrophotometer (AAS) Similar to previous studies the metals with the highest levels were K,

Al, Fe, Mn, and Zn. However, in Pakistani honey, Al was found in much lower amounts than the previous reports. On the basis of their results they have also concluded that the elemental composition of honey depends on soil composition, plant type, season, and environmental conditions ⁵. Different researchers in the world works on metal detection in different honey samples by AAS ¹²⁻¹⁴. Our study consists of the comparative metal detection in branded and non branded honey samples of different areas in Pakistan by AAS.

MATERIALS AND METHOD

Collection of Samples

Two types of honey samples (branded & non-branded) were collected from Islamabad, Charsadda, Peshawar, Swabi and Mardan in precleaned plastic bottles for quantitative determination of heavy metals (zinc, lead, copper, iron) levels.

Apparatus

The apparatus used is pH meter, conductivity meter, atomic absorption spectrophotometer (Perkin Elmer).

Table 1: The instrumental parameters for elements determination

Elements	Wavelength(nm)	Lamp current(A)	Slit width (cm)	Flame
Fe	248.8	5	0.2	Acetylene
Pb	283.3	5	0.2	Acetylene
Cu	357.9	6	0.5	Acetylene
Zn	213.9	7	0.4	Acetylene

Reagents

Concentrated nitric acid (65%) (HNO₃), Perchloric acid (HClO₄), Hydrogen peroxide (H₂O₂) and Distilled water. All the reagents were of analytical grade (Merck, Darmstadt, Germany). Double deionised water was used in all dilution procedures.

Experimental work

Physicochemical analysis

The physicochemical analysis like pH, ash content, moisture and electrical conductivity were determined by standard analytical methods ⁶.

Heavy metals detection

Ashing process

Approximately 5g of the required sample was weighted and transferred to the china dish. Then the total amount of 10mL conc. HNO₃ was added into it and it was properly mixed with the help

of a stirrer. Then the sample was taken and placed at approximately 150–200 °C on a hotplate for 30 minutes, in order to allow slow charring of the sample to occur. Then the sample was removed from hotplate as the brown fumes disappeared. Then it was treated with 2mL H₂O₂ or HClO₄, which were mixed properly, and again it was placed on a hotplate for further heating. The china dish was left on hotplate till to the complete evaporation of all volatile substances. Finally the sample was diluted with 25mL of distilled water, now the sample is ready for the analysis of metals under AAS.

Determination of elements

For the determination of each element by AAS, the instrument was set on recommended wavelength, burner position, as well as flame conditions were also used, as necessary for the analysis of elements. The spectrometer which is used was operated in the absorption mode, so it will give only those values which will be absorbed by the said sample. For these purpose three standard solutions for each element and a calibration curve was also prepared. The readings of concentrations of sample solutions after appropriate dilutions were then directly taken from the instrument. Distilled water was also atomized after each reading of standard or sample.

RESULTS AND DISCUSSION

The honey samples used in this work are divided into two categories. In category first are branded samples, which were procured from the market. These have been labeled as B. The second category of honey samples consists of non-branded samples labeled as NB.

In the present study, physicochemical analysis and four heavy metals namely Pb, Cu, Zn, and Fe were determined in honey samples. The reason for selecting these metals was because of their role in human health. The results obtained by using ashing process showed that Fe has a high value of 7.21 ± 2.01 and lowest value of 1.26 ± 0.05 , Cu has highest value of 0.17 ± 0.01 and lowest value of 0.02 ± 0.01 , Pb has highest value of 0.53 ± 0.03 and lowest of 0.07 ± 0.06 and Zn has a high value of 7.73 ± 0.02 and low value of 0.81 ± 0.13 . The results of physicochemical analysis and metals detection of the two categories of honey samples (NB and B Honey) are provided in tables 2, 3 and 4, 5 respectively. This method has provided comparable results.

Table 2 Physicochemical Analysis of non branded honey samples

Sample code	pH	Moisture (%)	Ash (g/100g)	Electrical Cond (mS/cm)
NB1	3.04	18.2	0.07	0.23
NB2	3.30	20.1	0.06	0.30
NB3	2.70	18.8	0.08	0.36
NB4	3.51	19.3	0.07	0.22
NB5	4.10	17.9	0.09	0.35

Table 3 Physicochemical Analysis of branded honey samples

Sample code	pH	Moisture (%)	Ash (g/100g)	Electrical Cond (mS/cm)
B1	4.05	18.5	0.04	0.31
B2	2.50	18.0	0.09	0.35
B3	3.21	17.8	0.07	0.20
B4	3.01	19.4	0.08	0.28
B5	3.45	18.2	0.09	0.30

Table 4 Concentration of heavy metals in non branded honey samples using ashing process

Sample code	Iron	Lead	Zinc	Copper
NB1	3.66±0.14	0.53±0.03	0.81±0.13	0.03±0.01
NB2	1.26±0.05	0.48±0.11	2.77±0.06	0.04±0.04
NB3	2.27±0.21	0.28±0.05	1.55±0.01	0.05±0.03
NB4	2.77±0.30	0.33±0.06	2.32±0.02	0.02±0.01
NB5	7.09±0.15	0.16±0.01	2.03±0.09	0.06±0.02

Table 5 Concentration of metals in branded honey samples using ashing process

Sample code	Iron	Lead	Zinc	Copper
B1	4.08±0.23	0.11±0.08	6.78±0.02	0.17±0.01
B2	3.80±0.71	0.32±0.03	4.01±0.19	0.08±0.03
B3	2.9±0.38	0.07±0.06	7.73±0.02	0.12±0.04
B4	6.32±1.38	0.14±0.02	3.41±0.04	0.10±0.06
B5	7.21±2.01	0.09±0.02	2.39±0.01	0.15±0.09

Values are expressed in milligrams per kilogram

CONCLUSION

The present study concludes that the heavy metals analysis of honey is essential for nutritional quality and safety of honey with regards to the contents of major, minor and trace elements. Of all the metals tested in the non branded and branded honey samples, honey from NB Charsadda have high contents of Fe, while the B honey samples from Charsadda have high content of Zn compared to the rest of the honey samples. While the Cu was found in lowest content in NB sample from Islamabad and B sample from district Mardan. The results of the present study reveal that the honey samples purchased from Charsadda, Mardan, Islamabad, Peshawar, and

Swabi have metal content below the safety baseline levels for human consumption.

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