



## PHYSIOCHEMICAL AND MINERAL ANALYSIS OF HONEY SAMPLES FROM VARANASI DISTRICT

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### ABSTRACT

Honey is a carbohydrate rich bioproduct produced by honeybees from the nectar of flowering plants. Composition of honey depends on the plant species visited by bees, environmental factors, processing and storage conditions. Honey contains sugar, proteins, moisture, vitamins, minerals, enzymes, polyphenols and flavonoides. While foraging, honey bees collect pollen along with the nectar and also receive minerals and heavy metals that plants get from soil, water and air. Determination of physiochemical properties, minerals and heavy metals is important for quality control and certification. Therefore, the present study aims to investigate physiochemical properties ( pH, EC, TDS, ash content, moisture content & colour), minerals (Ca, K, Na, Mg, Fe) and heavy metals (Zn, Pb, Cu, Cd, As) of six honey samples collected from Varanasi district. Among the physiochemical parameters mean values of pH ranged from 3.7 to 4.7, EC from 242±0.08 mS/cm to 1.15±0.16 mS/cm, TDS from 123.5±4.78 ppm to 576.6±81.15 ppm, moisture content from 13.38±0.11% to 21.44±0.21% and ash content 0.125±0.008% to 0.391±0.008%, while colour ranged from light extra amber to dark amber. Among the physiochemical parameters pH, TDS and ash content, were within the limit. EC in one sample and moisture in two samples were higher than the recommended limit by European Commission and Codex Alimentarius. Potassium was the most abundant mineral followed by sodium, calcium, magnesium and iron, Among the heavy metals lead, copper, cadmium, and arsenic were below the limit of detection while Zinc was within the permissible limits. Results indicate that the investigated honey samples are abundant source of minerals essential for human diet, growth and health and also provide indication of environment free from heavy metal in the investigated localities. The study explores good potential of Varanasi district to set up apiary industry for commercial honey production free from heavy metal.

**KEYWORDS:** Honey, minerals, heavy metals, physiochemical properties.



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## INTRODUCTION

Honey is a carbohydrate rich bioproduct produced by honeybees from the nectar of flowering plants. Composition of honey depends on the plant species visited by bees, environmental factors, processing and storage conditions.<sup>1-2</sup> In general honey contains sugar, proteins, moisture, vitamins, minerals, enzymes, polyphenols and flavonoids.<sup>3</sup> While foraging, honey bees also collect pollen grains along with the nectar and also receive minerals and heavy metals that plants get from soil, water and air. Contamination of plant tissue with heavy metals is hazardous for food chain and these accumulations cause dangerous effect on human health.<sup>4</sup> Thus the honey has been recognized as a biological indicator of environmental pollution.<sup>5</sup> Determination of physiochemical properties, minerals and heavy metals is important for the quality control and certification. Investigations on physiochemical and mineral contents of honey have been reported from various parts of the country.<sup>6-16</sup> However, to the best of our knowledge no information on physiochemical and mineral analysis of honey is available from Uttar Pradesh. Thus, the present investigation has been carried out to characterize honey samples collected from Varanasi district of Uttar Pradesh on basis of their physiochemical properties and minerals including heavy metals.

## MATERIALS AND METHODS

### *Study area and collection of materials*

Varanasi (latitude-25.3176 & longitude- 82.8739) is an old north Indian city on the banks of river Ganges in Uttar Pradesh, India. Varanasi is located at an elevation of 80.71 meters covering an area 1535 Sq. Km. Six squeezed honey samples of *Apis dorsata* were collected from six different localities viz. Manduadih (V1), Shivpur (V2), Chiraigaon (V3), Lalpur (V4), Danganj (V5) and Goverdhanpur (V6) of Varanasi district during January to March 2014. The samples were filtered with cloth to remove extraneous matter like bee wax pieces, scum and surface dirt and stored in airtight plastic bottles and labeled.

### *Physiochemical analysis*

Physiochemical analysis (determination of pH, Ash content, moisture) was done following methods recommended by Association of Official Analytical Chemists<sup>17</sup> pH was determined with digital pH meter (Labronics LT-49) from the solution of honey prepared by dissolving 10 gram of honey in 75 ml CO<sub>2</sub> free-distilled water. The pH meter was calibrated with buffer at pH 4, 7 and 9. For determination of ash content 3 gram of each sample was weighed in a silica crucible. The crucible was heated in a muffle furnace (Thermotech Tic-4000) for about 3 to 5 hours at 550°C. To ensure completion of ashing, it was reheated again in the furnace for half an hour (ash becomes white or grayish white). Ash was cooled in desiccators and weigh.

$$\text{Ash (\%)} = \frac{\text{Weight of sample after ashing}}{\text{Weight of fresh sample taken}} \times 100$$

For moisture content 3 gram sample materials were taken in a flat-bottom dish (pre-weighed) and kept overnight in a hot air oven at 100–110°C and weighed. The loss in weight was regarded as a measure of moisture content which was calculated by the following formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Electrical conductivity (EC) & Total dissolved solids (TDS) were determined with digital EC/TDS meter (Labronics LT-51) in honey solution made by dissolving 20 gram honey in 100 ml CO<sub>2</sub> free-distilled water.<sup>18</sup> Colour was determined with a spectrophotometer (Systronics: PC Based Dual beam spectrophotometer 2202) by reading absorbance at 635nm in a solution of 10g honey in 20 ml distilled water.<sup>19</sup> Table 1 shows honey colours, their absorbance and Pfund values obtained using the algorithm-  $\text{mmPfund} = -38.7 + 371.39 \times \text{Absorbance}$ .<sup>20</sup>

### *Determination of minerals and heavy metals*

Minerals (Ca, K, Na, Mg, Fe) and heavy metals (Zn, Pb, Cu, Cd, As) were determined in a certified laboratory (IIT, Mumbai) with the help of "Inductively Coupled Plasma-Atomic Emission Spectrometry" ICP-AES (Make: SPECTRO Analytical Instruments GmbH, Germany, Model: ARCOS, Simultaneous ICP Spectrometer).

## STATISTICAL ANALYSIS

All the data were taken in triplicates and analyzed using SPSS software version sixteen. The results were expressed as mean value with standard deviation. The significant differences were obtained by one way analysis of variance (ANOVA) followed by Duncan's multiple range test ( $P < 0.05$ ). Correlation coefficient were performed by Microsoft excel 2007.

## RESULTS AND DISCUSSION

### *Physiochemical analysis*

Results of physiochemical analysis viz. pH, EC, TDS, moisture content, ash content and colour are presented in Table 2. pH of honey is of great importance during extraction & storage as it influences the texture, stability and shelf life of honey.<sup>21</sup> In general honey is acidic in nature irrespective of its geographical origin, which is due to the presence of organic acids, particularly gluconic acid and inorganic ions such as phosphate &

chloride.<sup>7</sup> Standard range for pH of honey is considered to be between 3.2 to 4.5.<sup>22</sup> In the present investigation the mean pH values of the honey samples varied from 3.7 to 4.7. These values come under the range of pH values reported for various honeys from India (3.1 to 4.9)<sup>8-15</sup>, Algeria (3.49 to 4.6)<sup>23-25</sup>, Ethiopia (3.82 to 4.5)<sup>26</sup>, Brazil (3.22 to 4.19)<sup>27-28</sup>, Italy (3.50 to 4.21)<sup>29</sup> and Spain (3.56–4.79).<sup>30</sup> EC is one of the important parameter in determining the physical characteristic of honey. EC value depends on ash content, organic acid, protein content, and complex sugar and varies with botanical origin.<sup>21</sup> The origin of honey sample as blossom or honeydew is based on EC value as given by Codex Alimentarius Committee on Sugar and European Commission.<sup>32-33</sup> Generally, honey with EC value higher than the 0.8mS/cm is considered as honeydew honey while honey with lower values as blossom honey or blends of blossom with honey dew. In the present study mean EC value in the honey samples ranged from .242±0.08 to 1.15±0.16 mS/cm. Accordingly, sample V1 is honeydew honey, V2 blended and rest of the four sample (V3, V4, V5 and V6) are considered as blossom honey. TDS is the measurement of the combined inorganic and organic contents present in a honey sample. The mean values of TDS in the present investigation ranged from 123.5±4.78 to 576.6±81.15ppm (Table 2). Our study demonstrates that TDS shows positive correlation with EC as TDS increases EC also increases and vice-versa (Table 4). Moisture content of honey is an important factor contributing to its stability against fermentation & granulation during storage.<sup>6</sup> In the present study in four honey samples (V1, V2, V3 & V4) mean value for moisture content is within the acceptable range i.e. < 20%<sup>34</sup> while in two samples (V3 & V5) the moisture content is little higher (Table 2). Higher moisture content could lead to undesirable honey fermentation during storage caused by action of osmotolerant yeasts which results in the formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidised to acetic acid and water resulting sour taste of honey.<sup>35</sup> Similar little higher moisture content value were also reported up to 23.1 % and 27% in honey from Nagpur<sup>10</sup> and different region of Maharashtra<sup>31</sup>, respectively. In the present work mean values of ash content in honey varied from 0.1257±0.008% to 0.3910±0.008% (Table 2) which is within the acceptable range.<sup>36-37</sup> Variation in ash content could be due to harvesting practices used to separate

honey from comb, difference in soil types, atmospheric conditions as well as types and physiology of plants from where bees collect pollen/nectar.<sup>38</sup> Ash content values of honey samples showed a strong positive correlation with EC and TDS values (Table 4). Colour is the primary quality parameter of honey and is very important for commercial activity. Several factors can influence honey colour such as floral source, mineral content and storage conditions.<sup>39-40</sup> Colour of honey varies naturally ranging from light yellow to amber, dark amber and black in extreme cases and some time even green and red hues.<sup>41</sup> In the present study colour of honey was extra light amber in sample V1 & V2 (35.90 and 32.09 mmPfund), light amber in sample V3 and V5 (63.98 and 69.77 mmPfund ) amber in V6 (109 mmPfund) and dark amber in V4 (145.38 mmPfund), Figure 1.

#### **Mineral contents and heavy metals**

Mineral contents of honeys collected from different localities of Varanasi district along with the mean and correspondence standard deviation are given in Table 3. An analysis of variance for the mineral content results reveals that the average values are significantly different ( $P < 0.05$ ). Concentration of mineral and heavy metals in honey samples depends upon the nature of soil of source plants which in turn is greatly affected by atmospheric precipitation, use of pesticides and fertilizer in crop field.<sup>42</sup> In the present study concentration of selected minerals (K, Ca, Na, Mg, and Fe) in honey samples was determined. Among these, K was found to be most abundant mineral ranging from 1.803±0.07 to 47.668±0.07 ppm followed by Na (4.77±3.31 to 7.714±0.025 ppm), Ca (2.837±0.06 to 7.710±0.08 ppm), Mg (0.691±0.008 to 1.782±0.08 ppm) and Fe (0.046±0.005 to 0.647±0.05 ppm). In honey K being the most abundant mineral is also reported by other workers.<sup>43-47</sup> The Pearson correlation coefficients ( $p < 0.05$ ) between mineral contents and physiochemical parameters are presented in Table 4. All the minerals showed significant positive correlation with EC, TDS and ash content and a negative correlation with colour and moisture while with pH all minerals except Na & Fe showed a positive correlation. Among the heavy metals (Zn, Pb, Cu, Cd, As) Pb, Cu, Cd, and As were below the limit of detection (< 0.01ppm) while Zn was within permissible limit range (0.012±0.006 to 0.053±0.008ppm).<sup>48-50</sup>

**Table 1**  
**Honey colour expressed in absorbance and mmP fund.**

Honey colour	Absorbance	mmPfund
Water white	0.104-0.125	0-8
Extra white	0.125-0.148	8-16.5
White	0.148-0.195	16.5-34
Extra light amber	0.195-0.238	34-50
Light amber	0.238-0.333	50-85
Amber	0.333-0.411	85-114
Dark amber	>0.411	>114

**Table 2**  
**Statistical analysis of physicochemical properties of honey samples of Varanasi district.**

Parameters	V1	V2	V3	V4	V5	V6
Moisture content	17.58±0.372 <sup>c</sup>	13.38±0.117 <sup>a</sup>	21.44±0.215 <sup>e</sup>	17.22±0.117 <sup>b</sup>	21.22±0.073 <sup>e</sup>	18.186±0.101 <sup>d</sup>
Ash content	0.391±0.008 <sup>f</sup>	0.277±0.006 <sup>d</sup>	0.22±0.003 <sup>c</sup>	0.206±0.006 <sup>b</sup>	0.125±0.008 <sup>a</sup>	0.339±0.006 <sup>e</sup>
pH	4.72±0.106 <sup>c</sup>	3.71±0.094 <sup>a</sup>	3.9±0.244 <sup>a</sup>	4.31±0.140 <sup>b</sup>	4.27±0.083 <sup>b</sup>	3.82±0.240 <sup>a</sup>
EC	1.15±0.162 <sup>d</sup>	0.534±0.007 <sup>c</sup>	0.32±0.064 <sup>ab</sup>	0.32±0.024 <sup>ab</sup>	0.242±0.008 <sup>a</sup>	0.395±0.015 <sup>b</sup>
TDS	576.66±81.15 <sup>d</sup>	268±8.88 <sup>c</sup>	160.93±30.50 <sup>ab</sup>	173.23±10.97 <sup>ab</sup>	123.56±4.78 <sup>a</sup>	209±2.64 <sup>bc</sup>
Colour	Extra light amber	Extra light amber	Light amber	Dark amber	Light amber	Amber

Values are mean ± S.D.; n=3; EC= Electrical conductivity; TDS=Total dissolved solids.

Means are compared by using one way ANOVA-post Hoc Multiple comparisons' in each row, value with different letters (Superscripts) indicate significant differences (p<0.05).

**Table 3**  
**Statistical analysis of mineral contents (ppm) of honey samples of Varanasi district.**

Minerals	V1	V2	V3	V4	V5	V6
Ca	7.710±0.086 <sup>e</sup>	6.822±0.237 <sup>d</sup>	3.561±0.044 <sup>b</sup>	3.443±0.113 <sup>b</sup>	2.837±0.06 <sup>a</sup>	4.766±0.060 <sup>c</sup>
K	47.668±0.07 <sup>f</sup>	1.803±0.070 <sup>a</sup>	6.083±0.031 <sup>b</sup>	11.533±0.102 <sup>e</sup>	6.668±0.039 <sup>c</sup>	6.934±0.016 <sup>d</sup>
Na	7.458±0.189 <sup>b</sup>	6.804±0.066 <sup>ab</sup>	7.27±0.088 <sup>ab</sup>	4.77±3.312 <sup>a</sup>	6.273±0.120 <sup>ab</sup>	7.714±0.025 <sup>f</sup>
Mg	1.782±0.080 <sup>e</sup>	0.944±0.025 <sup>b</sup>	1.124±0.013 <sup>c</sup>	0.92±0.035 <sup>b</sup>	0.691±0.008 <sup>f</sup>	1.505±0.004 <sup>d</sup>
Fe	0.161±0.055 <sup>b</sup>	0.647±0.055 <sup>c</sup>	0.077±0.009 <sup>a</sup>	0.052±0.009 <sup>a</sup>	0.046±0.005 <sup>a</sup>	0.181±0.01 <sup>b</sup>
Zn	0.053±0.008 <sup>c</sup>	0.022±0.006 <sup>ab</sup>	0.023±0.006 <sup>ab</sup>	0.034±0.013 <sup>b</sup>	0.016±0.004 <sup>a</sup>	0.012±0.004 <sup>a</sup>
Pb	BLD	BLD	BLD	BLD	BLD	BLD
Cu	BLD	BLD	BLD	BLD	BLD	BLD
Cd	BLD	BLD	BLD	BLD	BLD	BLD
As	BLD	BLD	BLD	BLD	BLD	BLD

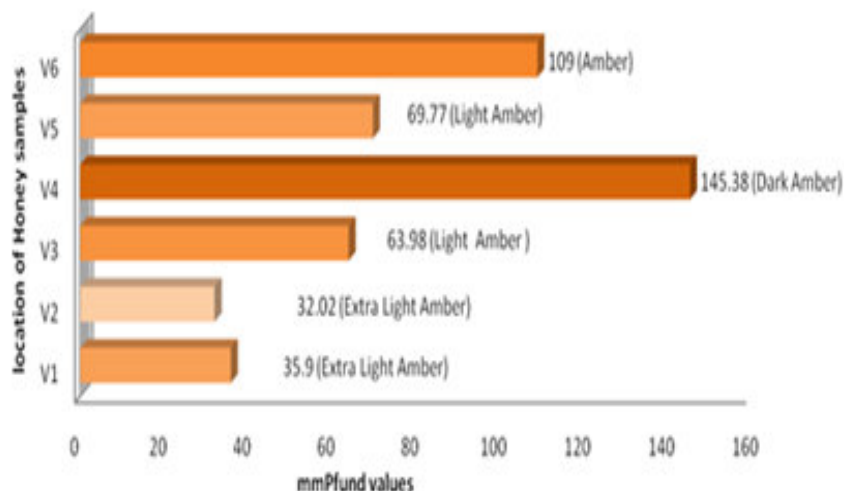
Values are mean± S.D.; n=3; BLD= below the limit of detection (0.01ppm)

Means are compared by using One way ANOVA-Post hoc multiple comparisons in each row, Value with different letters (superscript) indicate significant differences (p<0.05).

**Table 4**  
**Correlation among some physicochemical parameters and minerals**  
**(Pearson correlation coefficient, p<0.05)**

	pH	EC	TDS	Ash content	Moisture content	Colour (mmPfund)	Na	Mg	Fe	K	Ca	Zn
pH	1											
EC	0.587	1										
TDS	0.590	0.999	1									
Ash content	0.143	0.796	0.805	1								
Moisture content	0.204	-0.351	-0.357	-0.452	1							
Colour (mmPfund)	-0.014	-0.534	-0.512	-0.275	0.169	1						
Na	-0.217	0.405	0.399	0.615	0.090	-0.588	1					
Mg	0.320	0.774	0.783	0.930	-0.107	-0.194	0.654	1				
Fe	-0.513	0.207	0.200	0.314	-0.837	-0.528	0.226	-0.031	1			
K	0.847	0.906	0.909	0.618	-0.029	-0.288	0.222	0.731	-0.213	1		
Ca	0.171	0.878	0.876	0.835	-0.682	-0.627	0.477	0.654	0.636	0.597	1	
Zn	0.796	0.817	0.818	0.463	-0.197	-0.220	-0.093	0.507	-0.107	0.888	0.554	1

EC= Electrical conductivity, TDS= Total dissolved solids



**Figure 1**  
**Honey colour and mmpfund value of honey samples**

## CONCLUSION

Among the physicochemical parameters pH, TDS and ash content, are within the limit. EC in one sample and moisture in two samples is higher than the recommended limit by European commission and Codex Alimentarius. Regarding mineral contents honey sample V1 contains highest K, Ca, & Mg which may contribute to its high pH, EC, TDS and ash content. Honey sample V6 is rich in Na content while V2 is rich in Fe. Heavy metals Pb, Cu, Cd and As are below the limit of detection (0.01ppm) while Zn is within the permissible limit. Overall results indicate that the investigated honey samples are abundant source of minerals that are essential for human diet, growth and health and also provide an indication of environment free from heavy metals in the investigated localities of Varanasi district.

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The present study explores good potential of Varanasi district to set up apiary industry for commercial honey production free from heavy metal.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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