Physico-chemical analysis of stored and fresh honeys

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ABSTRACT

Honey is viscous and syrupy liquid has high nutritional and medicinal value and is extensively used in both Ayurvedic and Unani medicine. The composition of honey determines its value as nutritional and medicinal product and for that purpose physico-chemical analysis is carried out. In the present study different physical (pH, electrical conductivity, moisture contents, ash contents, refractive index, specific rotation) and chemical characteristics (nitrogen contents, hydroxy methyl furfural, free amino acids, total acidity, free acidity, lactone, fructose, glucose, fats and mineral elements) were determined in fresh and stored honey samples taken from Naran, Quetta and Rawalpindi. It was observed that the refractive index and specific gravity of the honey samples decreased with an increase in moisture content. All the samples were floral honey as they were levorotatory and moisture as well as ash contents of stored honey were less than the fresh ones. It contained traces of nitrogenous matter. Fresh honey contained small amounts of HMF as compared to the stored one while free amino acids of the stored one were found to be higher than that of fresh ones. Free acidity was same for all. Results of macro and microelements showed that K, Na, Ca and Mg were the macro-elements and Fe, Cu and Zn, microelements.

Key words: Honey, physico-chemical properties, metals.

INTRODUCTION

Honey is a natural, sweet, viscous liquid food produced in honey sacs of insect species of genus *Apis*, from nectar of flowers and to limited extent from juice of fruits and from honeydew. It was almost only source of sugar available to ancients and was valued for its medicinal and nutritional benefits (Crane 1976). It was an important commodity through out the ancient civilized world. It was favorite diet of the Holy Prophet (PBUH) as indicated by his saying:

"Honey is remedy for all illness of body and the Holy Quran is remedy for all illness of mind, Therefore, I recommend to you both remedies, the Holy Quran and Honey!"

Honey has high medicinal value. It helps to promote digestion and provides energy for muscle. It is used in treating ulcers, constipation, kidney problems, indigestion, bronchial infection, asthma, chronic disorders and sore throat. It is also used as a remedy for burns because of its anti-microbial activity. Appreciable increase in content of RBC and Hemoglobin is attributed to the presence of Fe, Cu, Mn and other elements in honey been taken. In spite of high sugar concentration, it can be given to diabetic patients. Honey dextran medium is used to preserve the cornea of the eye. Natural honey exhibits a very good performance as inhibitor for steel corrosion in high saline water, so also act as corrosion inhibitor (Abdullah 2000). The major components of honey are sugars (about 80%) and water (17-20%) and minor components include minerals, enzymes, lipids, amino acids, protein, organic acids etc. These minor elements determine its aroma, flavour and colour. The pleasant aroma and taste of this viscous liquid, ranging in colour from pale yellow to dark amber, also varies according to geographical and seasonal conditions (Rajalakshmi 1999).

The high content of sugars, small amount of amino acids, lipids along with some vitamins and minerals imparts its high nutritional value. It contains about 75-80% fructose that gives substantial energy and makes it sweeter than glucose or sucrose (Latif and Manzoor-ul-Haq 1956a). Vitamin content in honey is also very low but includes water-soluble vitamins: thiamine, riboflavin, ascorbic acid, pyridoxine pantothetic acid, biotin folic acid and nicotinic acid. It was reported to contain amino acids such as lysine, arginine, proline, methionine, iso-leucine and leucine along with aspartic acid, glutamate, serine, glycine, histidine and alanine. Honey has been reported to exert beneficial influence on retention of calcium and hence covers requirements from infants to elders. It is rich in carbohydrates and therefore is a wholesome food. It consists of nearly equal portion of glucose and fructose with water and small quantities of
nitrogenous matter and acids and occasionally sucrose and mannitol (Jabbar et al. 1996). Traces of alcohol are generally present. It also contains invertase and other enzymes derived from pollens. Due to certain specific characteristics like color, sweetness, viscosity, spread ability, miscibility, compatibility volume building and freezing point depression, honey is extensively used in bakery products, candies and confectionaries, cereal based car salad dressings, sauces, chocolates, fruit juices, puddings chilled desserts and microwave foods (Royden 1996).

The nutritional and medicinal importance of honey is directly related to its physico-chemical properties. The coloring matter present in honey includes chlorophyll, xanthophyll, anthocyanin, tannin and carotene. Darkening of honey either on heating or during storage is due to presence of amino acids in honey. Physical characteristics of honey (Bongdanov 1984; Jabbar et al. 1996) include pH, moisture content, ash contents, viscosity, specific gravity, surface tension, rheological properties, refractive index, thermal conductivity, freezing point depression etc. The chemical characteristics include the determination of nitrogen, hydroxy-methyl furfural, free amino acids, free acidity, lactone, fructose, glucose, fats and mineral elements.

In the present study physico-chemical analysis of honey samples produced in different areas of Pakistan was done in order to characterize and compare Pakistani honey quality with international standards.

**MATERIALS AND METHODS**

Three honey samples were taken from Narran, Quetta and Rawalpindi. The sample taken from Quetta was stored for approximately 2 years while the other two were fresh samples. Different physical characteristics including pH, moisture contents, ash contents, refractive index, electrical conductivity, specific rotation and the chemical characteristics involving nitrogen, hydroxy-methyl furfural, free amino acids (proline), total acidity, free acidity, lactone, fructose, glucose, fats and mineral elements were determined.

**Physical Characteristics**

Refractive index was determined by using Abbeye’s refractometer. For the determination of specific rotation, a polarimeter was used and specific rotation was calculated using formula:

$$\alpha = \frac{100\alpha \cdot \text{L} \cdot \text{C}}{L}$$

where $\alpha$ is the angle of rotation, $L$ is the length of polarimeter (in dm) and C is the concentration of honey solution. Moisture contents were determined by heating in Vacuum drying oven for overnight and maintaining pressure between 70-80 mm of Hg.

For the determination of ash content, honey samples were taken in pre-weighed crucible, heated till complete charring on a low flame and placed overnight in a muffle furnace at 550°C.

Electrical conductivity and pH was determined by using conductivity meter and pH meter, respectively.

**Chemical Characteristics**

Following methods were used for the chemical characteristics study of honey.

a) **Nitrogen**

Nitrogen in honey (Furnish and Tachell, 1989) was determined by digesting the sample in Kjeldahl’s flask using 20mL conc. H2SO4. Volume of the digested sample was made up and its 2mL were taken into Markham’s distillation apparatus, 5mL of 40% NaOH was added to it and titration flask containing boric acid was placed under condenser. The contents were heated by passing steam through apparatus until volume becomes 4 times. Then the solution in the flask was titrated against 0.1N HCl and percentage nitrogen was calculated.

b) **Hydroxy methyl furfural**

For the determination of HMF, 15% K3[Fe(CN)6] and 30% Zn(CH3COO)2 solution was added to the honey solution and filtered. 5mL of 0.3% NaHSO3 solution was added to the filtrate and mixed well. Absorbance of the sample was noted against reference at 284nm and 336nm. HMF was calculated by using formula:

$$\text{HMF} (\%) = \frac{(A_{284m} - A_{336m}) \times 14.97 \times V/W}{100}$$

where V is the volume of the filtrate taken and W is the weight of the honey.

c) **Free amino acid (Proline)**

To 0.5mL honey solution, taken in three borosilicate tubes, 1mL each of formic acid and ninhydrin solution was added. Placed on a water bath for 15 minutes and after cooling 5mL isopropyl alcohol was added and absorbance was noted at 520nm. Proline in honey was determined by comparing the values with
calibration curve values, plotted with standard solution of proline.

d) Total acidity, free acidity and lactone
Honey solution was made in CO₂ free water. After inserting pH electrode in solution, 0.05N NaOH was added till pH became 8.5. 10 ml more NaOH was added and back titrated against standard HCl to pH 8.5. Blank reading was taken in the same way. Free acidity, total acidity and lactone were determined by following formula:

Free acidity (meq/kg) = \((V_1-V_2) \times 50 / W\)
Lactone (meq/kg) = \((V_2-V_3) \times 50 / W\)
Total acidity (meq/kg) = Free acidity + Lactone

where \(V_1\), \(V_2\) and \(V_3\) are the volumes of NaOH used, \(V_3\) is the volume of HCl used and \(W\) is the weight of sample.

e) Fructose and glucose
Fructose and glucose in honey samples were determined by TLC by using silica gel as an adsorbent. Mixture of ethyl acetate, isopropanol and water (4:2:1) was used as solvent. Sugars are located by spraying the film with a solution of locating mixture (anisaldehyde and conc. H₂SO₄ in ethanol) and drying chrome plate to 100°C.

f) Fats
Fats were determined by cold extraction method using n-Hexane as solvent.

g) Mineral elemental study
For the determination of mineral elements (Na, K, Ca, Mg, Cu, Zn, Pb, Cd, Ni, Mn and Fe) acidic solution of ash was prepared with HCl and HNO₃ (3:1) and analyzed by Atomic Absorption Spectrophotometer using air-acetylene flame. Standard solutions of the elements were prepared by dissolving AR grade salts of the metals in 0.5N HNO₃ and diluting the stock solution to the desired range.

RESULTS AND DISCUSSION
The most common raw material for honey is nectar and other natural plant exudations. Nectar is a watery solution of sugars and originates in floral and extrafloral nectaries of plants. The composition of honey determines its value as nutritional and medicinal product and for that purpose physico-chemical analysis is carried out. Several properties like refractive index, density, viscosity, electrical conductivity, surface tension etc. are of great importance in honey industry as they influence its keeping quality, granulation and texture. Honey is basically composed of two important components i.e., moisture and sugars, along with various other minor components including minerals, HMF, amino acids, nitrogen etc.

Physical Characteristics

Refractive index
It is one of the important optical properties that provide an easy way of estimating the moisture content of honey, a value that determines its proneness to fermentations (Latif and Manzoor-ul-Haq 1956b). Fermentation of stored honey is uncommon. The refractive index of the honey samples decreased with increase in moisture contents at 20°C from 1.5044 (Sample 1) to 1.4840 (Sample 2). Refractive index of honey at 40°C also decreased from 1.4998 to 1.4794 (for Samples 1 and 2, respectively). Specific gravity decreases with the increase of moisture contents at 20°C. In the honey samples its value decreased from 1.4457 to 1.4794.

Specific Rotation
Honey has the property of rotating the plane of polarized light. This property depends largely on the sugars of honey, their types and relative proportion. Floral honeys are levorotatory. This is a consequence of normal preponderance in floral honey fructose, which has a negative specific rotation over glucose. The specific rotation of the samples was -15.00, -13.20 and -16.25.

Moisture and Ash
Moisture contents of the honey samples were approximately 14% and 16% (Table 1). It was also noted that the moisture contents of the stored honey were less than normal range. In the present study it was 12.81% for the stored sample. The ash contents of honey ranges from 0.24%-0.26% but ash contents of stored honey were less than normal range i.e. 0.14% only.

Electrical conductivity and pH
The electrical conductivity of honey determines its suitability for winter stores of bees. Krauze and Zalezewski (1991) reported the conductivity of different floral honey between 1.4 x 10⁻²-1.75 x 10⁻² mho/cm. In the present study the value (Table 1) ranged from 1.7 μohm/cm to 4.0 μohm/cm. pH of the samples taken from Narran, Quetta and Rawalpindi were 3.8, 4.0 and 3.6, respectively. This value is affected by amounts of various acids present, but mostly mineral
contents such as calcium, sodium, potassium and other constituent. Honey rich in ash generally showed higher pH values.

Table 1: Physical characteristics of honey samples

<table>
<thead>
<tr>
<th>Physical Characteristics</th>
<th>Sample 1 (Narran)</th>
<th>Sample 2 (Quetta)</th>
<th>Sample 3 (Rawalpindi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.8</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Electrical conductivity (μohm/cm)</td>
<td>1.8</td>
<td>4.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Moisture contents (%)</td>
<td>14.02</td>
<td>12.81</td>
<td>13.99</td>
</tr>
<tr>
<td>Ash contents (%)</td>
<td>0.2618</td>
<td>0.1469</td>
<td>0.2437</td>
</tr>
<tr>
<td>Refractive index (at 20°C)</td>
<td>1.5044</td>
<td>1.4840</td>
<td>1.4931</td>
</tr>
<tr>
<td>(at 40°C)</td>
<td>1.4998</td>
<td>1.4794</td>
<td>1.4830</td>
</tr>
<tr>
<td>Specific rotation</td>
<td>-15.00</td>
<td>-13.20</td>
<td>-16.25</td>
</tr>
</tbody>
</table>

Chemical Characteristics

a) Nitrogen

Honey usually contains traces of nitrogenous matter (Lothrop et al 1984) which is partially precipitated in colloidal forms and honey samples showed isoelectric point around pH 4.3. Nitrogen contents of samples taken from Narran, Quetta and Rawalpindi were 0.06%, 0.05% and 0.08%, respectively.

b) Hydroxy methyl furural

It is produced by the decomposition of fructose in the presence of acid. Fresh honey contains small amounts of HMF as compared to the stored one. The calculated value of HMF (Table 2) for sample 1 and 3 was 0.06% and 0.07%, respectively, while for the stored sample (taken from Quetta) the value was higher i.e. 0.08%. Bechik et al (1998) also reported that HMF content of honey does not increase during storage.

c) Free amino acids (Proline)

Amino acids are the breakdown product of proteins, which, in normal honeys, also exist in minute quantities, contributed by bee rather than plants. Proline is the most important of free amino acids. It value was found to be 9mg/100kg for sample 1 and 7mg/100kg for sample 3. Free amino acids of the stored sample (taken from Quetta) were found to be higher than the normal range i.e. 11mg/100kg. It was proved that darkening of honey during storage is due to high amount of free amino acids that produced dark melaninoid coloring matter by reacting with fructose and glucose.

d) Total acidity, free acidity and lactone

The level of acidity of honey contributes to its stability towards microorganisms. The bees increase acidity of honey during ripening. The complexity of honey extends to the number of acids present. Gluconic acid is present in considerable excess over all other acids. It is produced by the action of enzyme on dextrose present in honey. Gluconic acid exists in solution in equilibrium with lactone or internal ester. Total acidity is sum of acidity and lactone and was found to be 15, 13 and 15 meq/kg for sample 1, 2 and 3, respectively, while the value of free acidity was 10 meq/kg for all the three.

e) Fructose and glucose

It was reported (Ivanov 1997) that the quantity of predominate sugars varies with honey type from 31.01 to 41.44% for fructose and from 23.26 to 30.28% for glucose i.e. fructose was the dominate monosaccharide. The main disaccharides were turanose and maltose while sucrose was present in lower quantities. In the present study thin layer chromatography was used for the determination of sugar contents. Glucose and fructose are located after spraying locating agent and their Rf value was determined. The fructose glucose ratio was about
1.40 for the three samples of honey while the amount of sucrose present was 3, 4 and 2% for sample 1, 2 and 3, respectively.

f) Fats

Fats were found absent in all the three samples of Pakistani honey.

g) Mineral elemental study

The scientific literature on honey ash falls into three categories i.e. the amount of total ash, amount of principle constituents and the identities of minor metallic constituents, which often appear in minute amounts. Macro and microelements (K, Na, Ca, Mg, Zn, Ni, Cd, Cu, Mn, Fe, Pb) were determined by using atomic absorption spectrophotometer. It was noted that potassium (579.5, 470 and 687.9 ppm for sample 1, 2 and 3, respectively) and magnesium (126.26, 113 and 86.71 ppm for sample 1, 2 and 3, respectively) contents were higher than other macro elements. It was reported by (Lopez-Garcia et al 1999) that calcium content of the honeydew honeys was smaller than that of floral honeys. Similarly the amount of iron was higher (6.27, 3.81 and 6.30 ppm for sample 1, 2 and 3, respectively) than other microelements.

Table 3: Mineral elemental analysis of honey samples

<table>
<thead>
<tr>
<th>Mineral elements (ppm)</th>
<th>Sample 1 (Naran)</th>
<th>Sample 2 (Quetta)</th>
<th>Sample 3 (Rawalpindi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>579.50</td>
<td>470.00</td>
<td>687.90</td>
</tr>
<tr>
<td>Na</td>
<td>120.20</td>
<td>115.20</td>
<td>260.40</td>
</tr>
<tr>
<td>Ca</td>
<td>28.62</td>
<td>24.20</td>
<td>28.10</td>
</tr>
<tr>
<td>Mg</td>
<td>126.26</td>
<td>113.00</td>
<td>86.71</td>
</tr>
<tr>
<td>Zn</td>
<td>6.57</td>
<td>7.79</td>
<td>11.46</td>
</tr>
<tr>
<td>Ni</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Cd</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Cu</td>
<td>0.28</td>
<td>0.73</td>
<td>0.69</td>
</tr>
<tr>
<td>Mn</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Fe</td>
<td>6.27</td>
<td>3.81</td>
<td>6.30</td>
</tr>
<tr>
<td>Pb</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Results of macro and microelements (Table 3) showed that honey samples contains potassium, sodium, calcium and magnesium as macro-elements and iron, copper and zinc as microelements while manganese, nickel, cadmium and lead were absent in all the three samples. Iron is essential constituent of hemoglobin, which carries oxygen to our body tissues and copper promotes action of iron. Calcium is essential for bones and teeth. It was present in considerable amounts (28.62, 24.20 and 28.10 ppm for sample 1, 2 and 3, respectively).

CONCLUSIONS

The nutritional and medicinal importance of honey is directly related to its physico-chemical properties. Usually analysis of honey showed considerable differences according to their place of origin, but the three samples studied, showed insignificant difference in values of almost all the physico-chemical characteristics. Fresh honey contained small amounts of HMF as compared to the stored one while free amino acids of the stored one were found to be higher than that of fresh ones. Results of macro and microelements showed that honey samples contains potassium, sodium, calcium and magnesium as macro-elements and iron, copper and zinc as microelements while manganese, nickel, cadmium and lead were absent in all the three samples.

REFERENCES


