Full Length Research Paper

A study on the determination of physicochemical properties of honey from different valleys of Gilgit-Baltistan

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This study was carried out to investigate the physicochemical properties of local honey collected from different flora of Gilgit-Baltistan namely: Herbal, Acacia and Berry, promoted by some local honeybee keepers who are supposed to be famous for quality honey produce. The physicochemical parameters like moisture content, pH, titratable acidity, total sugars, reducing sugar and non-reducing sugar were analyzed. Research findings pointed out that the physicochemical property in local honey of Gilgit was in accordance with the codex standard and it meets the significant quality criteria of a high-quality honey. Besides, the sensorial excellence of local honey like color, texture and flavor were also significantly superior to any other honey produced in any other corner of Pakistan. It is pertinent to declare that necessary precautions must be taken to ensure standardized beekeeping techniques, manufacturing and storing processes to improve honey quality.

Key words: Honeybees, flora of Gilgit-Baltistan, chemical properties.

INTRODUCTION

Both the Holy Qur’an and Hadith refer to honey as a healer of disease. And thy Lord taught the bee to build its cells in hills, on trees and in (men's) habitations. There issues from within their bodies a drink of varying colors, wherein is healing for mankind. Verily in this is a sign for those who give thought’ (Translation of Quran 16:68-69).

Besides, the prophet (PBUH) said: Honey is a remedy for every illness and the Qur'an is a remedy for all illness of the mind, therefore I recommend to you remedies, the Qur'an and the honey (Bukhari).

Honey was used from ancient time both as a natural sweetener and a healing agent (National Honey Board, 2002). The composition and flavor of honey varies, depending mainly on the source of the nectar(s) from which it originates and to a lesser extent on certain external factors - climatic conditions and beekeeping practices in removing and extracting honey (White, 1975a). Chemical composition of honey mainly depends on the vegetation sources from which it derives, though external factors like climate, harvesting conditions and storage can also influence it (Crane, 1980). Careless handling of honey can reduce its quality. Honey is a complex natural product produced by honeybees from the nectar of blossoms or from exudates of trees and plants, usually with the participation of plant-sucking insects. These different botanical origins give rise to nectar or honeydew honeys respectively. Honey has been used since ancient times mainly as a sweetening agent, but it has also been employed in a therapeutic capacity. The high sweetening power of honey is due to the presence of the monosaccharides’ fructose and glucose as main components (60 to 85%); however, the

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components involved in its therapeutic properties have not yet been determined. Thus honey is a very complex product; its composition is closely associated with its botanical origin, with phenolic compounds, minerals, proteins, free amino acids, enzymes and vitamins as minor components (Ortiz et al. 1989).

There are three families of social bees which produce honey; these are: the Bombiidae, Meliponidae and Apidae (Smith et al., 2009). The Bombiidae are found mainly in temperate climates. Their nests are very small, often in the ground and they have no commercial importance except as pollinators of certain plants. The family Apidae, to which the honeybee belongs, is indigenous only to Europe, Africa and Asia (FAO, 1986; Crane, 1990). About 9 species of honeybees have been recognized in the world (Roubik, 1989). These are *Apis andreniformis, Apis cerana, Apis cerana indica, Apis dorsata, Apis dorsata binghami, Apis florea, Apis laboriosa, Apis mellifera* and *Apis vechti*. Among these, the following are the major honeybee species and are of world economic importance: *Apis cerana/indica, Apis dorsata, Apis florea* and *Apis mellifera*. The bees reared in Gilgit are mostly belonging to the specie Apidae and sub specie *Apis mellifera*. The quality and verity of honey is mostly dependent on the different types of plant nectars and flowers available in the area where the bee colonies are lift. In Gilgit-Baltistan, there is mostly three seasons: acacia flowering season (February to March) Russian olive (Berry) flowering season (April to June), and Herbal season (July to September). Thus, the honey is divided into three varieties on the basis of seasonal differences like Acacia, Berry and Herbal.

The precise composition of honey varies according to the plant species on which the bee forages but the main constituents remain the same in all honeys. Keeping in mind the compositional differences in honey due to flora and climatic change in the in-depth valleys of Gilgit-Baltistan, this study was conducted with the objectives to classify the best local variety, to find out the sensorial excellence and to determine the physicochemical properties.

**MATERIALS AND METHODS**

This study was carried out in the Department of Agriculture and Food Technology, Karakorum International University, Gilgit. It aims to analyze the physicochemical properties of honey varieties produced in various parts of District Gilgit. Honey samples were collected from local producers famous for pure honey production. The collected samples were safely transported from different remote valleys to the laboratories of the Department of Agriculture and Food Technology, Karakorum International University, Gilgit for analysis.

**Determination of moisture content**

The moisture content was determined according to AOAC (2000). In this regard, the sample materials were taken in a flat-bottom dish (pre-weighed) and kept overnight in an 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:

\[
\text{Moisture (\%)} = \frac{\text{Weight of fresh sample – Weight of dry sample}}{\text{Weight of fresh sample}} \times 100
\]

**Determination of ash content**

For determination of ash content, the method of AOAC (2000) was followed. According to the method, 10 g of each sample was weighed in a silica crucible. The crucible was heated in a muffle furnace for about 3 to 5 h at 500°C. It was cooled in desiccators and weighed. To ensure completion of ashing, it was reheated again in the furnace for half an hour more, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or grayish white). Weight of ash gave the ash content and was calculated by the following formula:

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

**Determination of titratable acidity**

Titratable acidity as tartaric acid was determined according to the method of AOAC (2000). Each sample of the honey was treated with 0.1N NaOH solution using titration kit, where 3 to 5 drops of phenolphthalein indicator were used. The volume of alkali used was noted and calculated by using the following formula:

\[
\text{Titratable acidity (\%)} = \frac{1 \times \text{Eq. Wt. of acid} \times \text{Normality of NaOH} \times \text{titer}}{10 \times \text{Wt. of sample (g)}} \times 100
\]

**Determination of total soluble solids**

The total soluble solids (TSS) were determined as per the method described by Mazumdar and Majumder (2003) using Digital-Bench-Refrectometer. Before use, the instrument was cleaned and adjusted to zero at 20°C using distilled water. An appropriate quantity of sample of each variety of honey was placed on the prism-plate of the Refrectometer with the help of a glass rod and folding back the cover. For each sample, the instrument was calibrated using distilled water. The reading that appeared on the screen was directly recorded as total soluble solids (Brix).

**Determination of pH**

For determination of pH in the honey, the method of AOAC (2000) was adopted and digital pH meter was used. The pH meter was calibrated with buffers at pH 4.
Table 1. Chemical analysis of local honey of District Gilgit.

<table>
<thead>
<tr>
<th>Variety</th>
<th>% moisture</th>
<th>% Ash</th>
<th>% Acidity</th>
<th>pH</th>
<th>% TSS</th>
<th>% Total Sugar</th>
<th>% Reducing sugar</th>
<th>% Non-reducing sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbal</td>
<td>18.2*</td>
<td>0.074*</td>
<td>0.492*</td>
<td>3.2*</td>
<td>75*</td>
<td>73.06*</td>
<td>69*</td>
<td>4.05*</td>
</tr>
<tr>
<td>Acacia</td>
<td>18.6*</td>
<td>0.054*</td>
<td>0.426*</td>
<td>3.4*</td>
<td>76*</td>
<td>79.1*</td>
<td>75*</td>
<td>4.1*</td>
</tr>
<tr>
<td>Berry</td>
<td>16.4*</td>
<td>0.082*</td>
<td>0.320*</td>
<td>3.5*</td>
<td>77*</td>
<td>73.6*</td>
<td>68*</td>
<td>5.6*</td>
</tr>
</tbody>
</table>

Values were obtained after triplicate analysis, *Significance at P < 0.05.

Table 2. Sensory evaluation of local honey of District Gilgit.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Herbal</th>
<th>Acacia</th>
<th>Berry</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7.6*</td>
</tr>
<tr>
<td>Flavor</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7.6*</td>
</tr>
<tr>
<td>Mouth feel</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>7.0*</td>
</tr>
<tr>
<td>Hardness</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6.5*</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7.6*</td>
</tr>
</tbody>
</table>

Scores were obtained after triplicate replications, *Significance at P < 0.05.

and 10. Sample solution was taken in the beaker and inserted. When the first reading was completed, the electrode was washed with distilled water and dried-up with tissue paper. Similarly, as a continue series, all other samples were determined accordingly.

**Determination of sugars**

Determination of sugars (total sugar, reducing sugar and non-reducing sugar) was carried out through Lane and Eynon method as described by James (1995).

**Total sugars and reducing sugars**

For this solution, 5 g of sample was taken into a beaker and 100 ml of warm water was added to it. The solution was stirred until all the soluble matters were dissolved and filtered through Whatman filter paper into a 250 volumetric flask. 100 ml of the solution was pipetted and prepared into a conical flask, after which 10 ml of diluted hydrogen chloride (HCl) was added and boiled for 5 min. On cooling, the solution was neutralized to phenolphthalein with 10% NaOH and kept in a 250 volumetric flask. This solution was used for titration against Fehling’s solution and the reading was calculated as follows:

$$\text{Total sugar} \% = \frac{\text{Factor (4.95) \times dilution (250) \times 2.5}}{\text{Titre \times wt of sample \times 10}}$$

$$\text{Reducing sugar} \% = \frac{\text{Factor (4.95) \times dilution (250)}}{\text{Titre \times wt of sample \times 10}}$$

**Non-reducing sugar**

It was estimated as the difference between the total sugar content and reducing sugar content on subtraction (total sugar-reducing sugar).

**Statistical analysis**

The results were analyzed using SPSS statistical program version fourteen. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities (SPSS). For statistical data, standard descriptive statistics were performed for each of the quantitative parameters.

**RESULTS AND DISCUSSION**

Tables 1 and 2 show the chemical analysis of local honey of District Gilgit, and the sensory evaluation of local honey of District Gilgit, respectively.

Moisture content is one of the most important compositions to be considered as a quality parameter of honey. The maximum moisture content was found to be 18.6% in Acacia samples, whereas 18.2% moisture content was observed in Herbal variety. These results are in agreement with the findings of Cantarelli et al. (2008) who reported that the moisture content in honey was recorded in the range of 14 to 18%; however it depends upon the season and geographic condition. Furthermore, these results are also in agreement with those of Nuru (1999) and Downey et al. (2005) who reported that the range of moisture content of pure honey is 16.10 to 23.36%. Fredes and Montenegro (2006) reported that honey containing lower moisture content will have a longer shelf life. The maximum ash content (0.082%) was found in Berry followed by Acacia (0.054%), whereas Herbal variety was 0.074%. These findings are in agreement with those of Ihtisham-ul-haq (1997) who...
analyzed different varieties of honey for determination of ash content and draw a range of 0.008 to 0.49% ash in honey samples. These results are further in agreement with those of White (1975a) who worked on different varieties of honey and obtained ash content in the range of 0.020 to 1.028%. The variation may be due to many factors such as soil conditions, atmospheric conditions and physiology of each plant.

Results indicate maximum acidity of 0.49% in Herbal variety followed by Acacia and Berry varieties in decreasing order with 0.42 and 0.32% respectively. The total acidity of all the samples analyzed was found within the corresponding limits of 0.04 to 0.55% as described by Codex Alimentarii Commission (2001). Besides, White et al. (1962) reported free acidity of 0.022%, whereas total acidity was determined as 0.29% in 490 samples of honey. Ihtisham-ul-haqq (1997) reported similar results in comparison to those of this study. Furthermore, the maximum pH value of 3.4 was found in Acacia variety whereas 3.5 and 3.2 was determined in Berry and Herbal varieties respectively. These observations are in accordance with those made by Codex Alimentarii Commission (2001) where acceptable ranges of pH of honey were predetermined between 3.2 and 4.5. The results of this study are also in agreement with those of Hussain (1989) who reported the pH of 3.0 to 5.0 in pure honey. These pH ranges are mainly due to the variation of different acid and minerals present in the honey. Likewise, the floral difference may also cause the ranges of pH.

Results of this study illustrate the concentration of sugars of which total sugar was 79.1, 73.06 and 73.6% in Acacia, Herbal and Berry varieties respectively, whereas reducing sugar was determined as 75% in Acacia, 69% in Herbal and 68% in Berry varieties of honey. These results can be compared with the findings of Kamal et al. (2002) who reported 77 and 71% total sugar in different varieties of honey. In addition, many other scientists such as Castilla (1996) and Joshi (1997) also reported closely related findings of total sugar ranging from 53.3 to 80.7% in different varieties of honey. The results of this study on reducing sugar are also in agreement with the findings of Latif et al. (1956) who reported 65 to 76% in different varieties of honey. The results of reducing sugar are in agreement with those of Abu-Taoush et al. (1993) who reported 79.46% reducing sugar in a specific variety of honey. Likewise, non-reducing sugar was determined to be 5.6% as maximum concentration in Berry, whereas it was 4.05% in Herbal variety and 4.1% in Acacia variety. The findings of this study are further authenticated by Codex Alimentarius Commission (1969) given the fact that the range of non-reducing sugar in honey is 1.15 to 12%. Besides, these results are also in agreement with those of Kamal et al. (2002) who indicated a range of 1.115 to 12.135% in honey varieties. Total Soluble Solids (TSS) was found as 77% for Berry variety, whereas the other two varieties, Acacia and Herbal, showed 76 and 75% TSS respectively. White (1975a) also agreed with these findings and report that honeys contain 73 to 79% TSS (brix). Organoleptical evaluations indicate that Acacia variety of honey obtained more scores than the other two, that is, Berry and Herbal. Besides, the sensorial analysis indicates that honey produced from Gilgit-Baltistan have a superior quality in terms of color, flavor, mouth feel and overall acceptability. However, its hardness is little more which may be due to the cold environment of the region.

Conclusion

Although the honeybee samples collected from retailers of different valleys of Gilgit-Baltistan were found to have good quality physicochemical characteristics, the beekeepers should be educated for further improvement. However, further studies are needed in order to determine its hardness whether this hardness is associated with natural sugars or some other environmental reasons.

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