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**BIO-CHEMICAL EVALUATION OF HONEY PRODUCED BY *APIS MELLIFERA* HONEYBEE COLLECTED FROM JHANG, BAHAWALPUR, MULTAN, JHELUM AND KALAR KAHAR AREAS**

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**ABSTRACT:-** The values of various quality criteria of Pakistani honeys were found within the permissible limits. The average moisture contents were 18.30%, 18.35%, 17.66%, 16.27% and 18.7% from Jhang, Bahawalpur, Kalar Kahar, Multan and Jhelum, respectively. Similarly, the pH 4.26, 4.50, 3.91, 3.72 and 3.33; electrical conductivity, 204.67 mScm<sup>-1</sup>, 137.8 mScm<sup>-1</sup>, 202.62 mScm<sup>-1</sup>, 277.33 mScm<sup>-1</sup> and 196.96 mScm<sup>-1</sup>; free acidity 25.80 meqkg<sup>-1</sup>, 26.16 meqkg<sup>-1</sup>, 24.97 meqkg<sup>-1</sup>, 30.25 meqkg<sup>-1</sup>, 25.4 meqkg<sup>-1</sup>; lactone 8.17 meqkg<sup>-1</sup>, 6.52 meqkg<sup>-1</sup>, 6.68 meqkg<sup>-1</sup>, 5 meqkg<sup>-1</sup> and 4.08 meqkg<sup>-1</sup>; total acidity, 32.96 meqkg<sup>-1</sup>, 32.52 meqkg<sup>-1</sup>, 31.65 meqkg<sup>-1</sup>, 35.25 meqkg<sup>-1</sup> and 28.55 meqkg<sup>-1</sup>; proline content, 376.64 mgkg<sup>-1</sup>, 312.8 mgkg<sup>-1</sup>, 260.17 mgkg<sup>-1</sup>, 402.57 mgkg<sup>-1</sup> and 286.84 mgkg<sup>-1</sup>. Hydroxymethylfurfural (HMF) content of honey samples were measured as 30.25 mgkg<sup>-1</sup> from Jhang, 18.10 mgkg<sup>-1</sup> from Kalar Kahar, 25.29 mgkg<sup>-1</sup> from Bahawalpur, 19.71 mgkg<sup>-1</sup> from Multan, and 19.62 mgkg<sup>-1</sup> from Jhelum, respectively.

*Key Words: Apis mellifera; Honey; Physicochemical Characteristics; Proline Content; HMF Content; Pakistan.*

## INTRODUCTION

Honey is one of the most complex mixtures of carbohydrates and other minor components produced in nature. It is an energy food that is used as an ingredient in many manufactured foods, mainly, cereal - based products, for its sweetness, color, flavor, caramelization, pump ability and viscosity (La Grange and Sanders, 1988; Qamer et al., 2008). Honey is a natural product that is widely available all over the world and is readily obtained from natural or cultured bee colonies (Terrab et al., 2002; Yao et al., 2003; Sanz et al., 2003; Malacalza et al., 2005). Generally honey is evaluated by a

physicochemical analysis of its constituents. Several of these are of great importance to the honey industry as they influence the storage quality, granulation texture, flavor, nutritional and medicinal value of the honey. Its physical properties and chemical composition from different sources have been published by many scientists (Sporns et al., 1992; Perez - Arquillue et al., 1995; Singh and Kauser, 1997; Qamer et al., 2005). The composition of honey depends highly on the type of flowers utilized by the bee as well as climatic conditions (Abu-Tarboush et al., 1993; Iglesias et al., 2004; Qamer et al., 2005).

The present study has been

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conducted to find the *Apis mellifera* colonies at different localities, to increase the scientific knowledge about composition of its honey in Pakistan and further evaluate its physicochemical properties.

### MATERIALS AND METHOD

Twenty five honey samples were obtained from beekeepers of different areas of Pakistan i.e., Jhang, Bahawalpur, Kalar Kahar, Multan and Jhelum. These samples were collected during different seasons of the year depending on floral source, packed in glass jars labeled with site information and taken to the laboratory. There was no sign of granulation in any sample. No preservative or heating was applied during analysis. Honey samples were diluted in distilled water at room temperature (25-29°C) according to the requirements of the tests. Experiments were conducted in the Research Laboratory of Zoology Department Government College University, Faisalabad. All physicochemical determinations were essentially carried out according to the European Honey Commission methods (Bogdanov et al., 1999). The data was subjected to statistical analysis in which different physicochemical parameters of honey were compared using analysis of variance (Steel and Torrie, 1981).

### RESULTS AND DISCUSSION

#### pH

The average pH 4.26 (3.75-4.93) from Jhang and 4.50 from Bahawalpur were found close to the values reported by Thrasyvoulou and Manikis (1995) in pine (4.6), fir (5.0), chestnut (4.9) and Erica (4.2) honeys

respectively. The average pH of 3.91(3.64-4.08) from Kalar Kahar and 3.72 from Multan were found close to the pH (3.91) reported by United States Department of Agriculture (1951). Gomez et al. (1993) and Luis Carilous (2007) reported pH of 3.62 and 3.19 in Eucalyptus and orange honey, respectively. Iglesias et al. (2004) reported pH of 3.9 in honey samples obtained from various floral sources. Qamer et al.(2005) recorded pH of 3.75 in ber honey samples produced by *Apis mellifera*. It verified the statement of Abu-Tarboush et al. (1993) that variation in the pH is due to different plant sources. The current pH values obtained from *A. mellifera* multi-floral honey were nearby as reported by Terrab et al. (2002) (4.77); Meda (2005) (3.5-4.7); Baratakova (2007) (3.29-4.05); Osman et al. (2007) (3.88-4.58) and Rural (2008) (3.48-3.83).

#### Moisture Contents

The average 18.3%, 18.35% and 18.7% moisture content from Jhang, Bahawalpur and Jhelum, respectively, were almost similar as reported by Thrasyvoulou and Manikis (1995) in Erica (18.6%). However, less than 18.8% from 128 wild honey samples tested by Luis Carilous et al. (2007). Anupama et al. (2003) reported 17-22.6% moisture content. The average water content of 17.66% from Kalar Kahar was slightly higher than 17.2% stated by Joshi et al. (2000), while low in sunflower and orange (17.7%, 17.82%, respectively) honeys described by Qamer et al. (2005) and Luis Carilous et al. (2007), respectively. Minch et al. (1971) reported much higher i.e., 20.7% water in the Philippines honey samples of same

honeybee species. Moisture content (16.26%) from Multan honey samples seems same as assessed by Qamer et al. (2005) (16.2%) in Phulai and Thrasyvoulou and Manikis (1995) in pine (16.6%), chestnut (16.3%), thyme (16.1%) and orange (16.9%) honey samples. The moisture content in all *A.mellifera* honey samples ranged from 16.5% to 20.5% as already given by Ciappini et al. (2002); 22.2% (Meda et al., 2005; Khan et al., 2006) 15.60-19.2%; 14.45-15.95% (Osman et al., 2007); 15.0-20.0% (Bartakova, 2007) and 16.5-19.2% (Rural, 2008). In general, high moisture content causes the honey to ferment, spoil and lose flavor, thus causing honey quality loss (Costa et al., 1999). As far as the moisture contents of the analyzed honey samples are concerned, they are in the limits of International Honey Standards and of Directive 2001/110/EC from Council of European Union (2001), according to which water content of honey should not be more than 21%.

#### **Electrical Conductivity (EC)**

The mean electrical conductivity recorded by Joshi et al. (2000) and Rural (2008) seems similar to the EC of honey samples from Jhang, Kalar Kahar, Multan and Jhelum which were 373.4-218.4 mS cm<sup>-1</sup>. On the other side, EC of all honey samples collected from various areas of Punjab were higher than shown by Thrasyvoulou and Manikis (1995) in pine (1.26 mS cm<sup>-1</sup>), fir (1.40 mS cm<sup>-1</sup>), chestnut (1.54 mS cm<sup>-1</sup>), thyme (0.42 mS cm<sup>-1</sup>), cotton (0.60 mS cm<sup>-1</sup>), sunflower (0.43 mS cm<sup>-1</sup>), orange (0.19 mS cm<sup>-1</sup>) and Erica (0.67 mS cm<sup>-1</sup>). These EC values for Pakistani honey

samples were within the limits of International Honey Standards and Directive 2001/110/EC from Council of European Union (2001) for blossom honey (0.8 mS cm<sup>-1</sup>).

#### **Free Acidity (FA)**

The estimated free acidity (24.97-26.76 meq kg<sup>-1</sup>) from Jhang, Bahawalpur, Kalar Kahar and Jhelum are approximately in the same range (25.34 meq kg<sup>-1</sup>) demonstrated by Gomez et al. (1993), 8.6-45.9 meq kg<sup>-1</sup> (Ciappini et al. 2002) and 23.55-58.52 meq kg<sup>-1</sup> by Khan et al. (2006). Honey samples from Multan showed 30.25 meq kg<sup>-1</sup> FA that seems close to Eucalyptus honey acidity (33.85 meq kg<sup>-1</sup>) reported by Luis Carilous et al. (2007). However, Qamer et al. (2005) described low FA range of 5.5-6 meq kg<sup>-1</sup> in sunflower, Phulai and ber honeys produced by *Apis mellifera*. The free acidity of all honey samples collected from different areas of Punjab Province was less than 50 meq kg<sup>-1</sup>, a maximum limit for acidity prescribed by International Honey Standards and by the Directive 2001/110/EC from the Council of European Union (2001).

#### **Lactone Content (LA)**

Qamer et al. (2005) analyzed the fresh honey samples from the nectar sources of Phulai (1 meq kg<sup>-1</sup>), sunflower (0 meq kg<sup>-1</sup>) and sidder (0.51 meq kg<sup>-1</sup>) and reported much less lactone content as compared to the content determined in the present study. Lactone content were measured at an average of 8.17 meq kg<sup>-1</sup> in honey samples collected from Jhang, 6.52 meq kg<sup>-1</sup> from Bahawalpur, 6.68 meq kg<sup>-1</sup> from Kalar Kahar, 5.00 meq kg<sup>-1</sup> from Multan and 4.08 meq kg<sup>-1</sup> from Jhelum.

### Total Acidity (TA)

It is the sum of free and lactone acidity. The average total acidity assessed was  $32.96 \text{ meq kg}^{-1}$  ( $26.33\text{-}37.83 \text{ meq kg}^{-1}$ ) from Jhang,  $32.52 \text{ meq kg}^{-1}$  ( $27.17\text{-}36.5 \text{ meq kg}^{-1}$ ) from Bahawalpur,  $31.65 \text{ meq kg}^{-1}$  ( $27.17\text{-}39.44 \text{ meq kg}^{-1}$ ) from Kalar Kahar,  $32.25 \text{ meq kg}^{-1}$  from Multan and  $28.55 \text{ meq kg}^{-1}$  from Jhelum were found within the range of  $26.1 \text{ meq kg}^{-1}$  recorded by Minch et al. (1971) in Philippines honey samples. However, Gomez et al. (1993) noted  $28.28 \text{ meq kg}^{-1}$ ; Osman et al. (2007)  $10.90\text{-}21.84 \text{ meq kg}^{-1}$  and Rural (2008)  $22\text{-}40 \text{ meq kg}^{-1}$  total acidity in honey samples.

### Proline Content

A variation in proline content has been noticed by Thrasyvoulou and Manikis (1995) from 174 samples of pine ( $514 \text{ meq kg}^{-1}$ ), fir ( $390 \text{ meq kg}^{-1}$ ), chestnut ( $554 \text{ meq kg}^{-1}$ ), thyme ( $790 \text{ meq kg}^{-1}$ ), cotton ( $432 \text{ meq kg}^{-1}$ ), sunflower ( $665 \text{ meq kg}^{-1}$ ), orange ( $326 \text{ meq kg}^{-1}$ ) and Erica ( $536 \text{ meq kg}^{-1}$ ). The average calculated proline content in honey samples collected from Jhang ( $378.62 \text{ mg kg}^{-1}$ ) and Jhelum ( $286.4 \text{ mg kg}^{-1}$ ) were higher ( $466.2\text{-}644 \text{ mg kg}^{-1}$ ) than maximum values reported by Qamer et al. (2005) in Phulai and sunflower honeys. However, this content in Bahawalpur ( $1032 \text{ mg kg}^{-1}$ ), Kalar Kahar ( $2252.20 \text{ mg kg}^{-1}$ ) and Multan ( $2409.35 \text{ mg kg}^{-1}$ ) were less than reported by Qamer et al. (2005) in ber honey samples ( $2800 \text{ mg kg}^{-1}$ ) produced by *Apis mellifera*. Gomez et al. (1993) and Joshi et al. (2000) found proline content of  $46.4 \text{ mg kg}^{-1}$  and  $610 \text{ mg kg}^{-1}$ , respectively.

### Hydroxymethylfurfural (HMF) Content

The average HMF content were

$30.7 \text{ mg kg}^{-1}$  ( $30.78\text{-}63.87 \text{ mg kg}^{-1}$ ) in honey samples collected from Jhang,  $25.29 \text{ mg kg}^{-1}$  ( $28.86\text{-}80.267 \text{ mg kg}^{-1}$ ); Bahawalpur,  $18.19 \text{ mg kg}^{-1}$  ( $53.82\text{-}65.24 \text{ mg kg}^{-1}$ ) Kalar Kahar,  $19.7 \text{ mg kg}^{-1}$  ( $64.3\text{-}85.21 \text{ mg kg}^{-1}$ ) Multan and  $19.62 \text{ mg kg}^{-1}$  from Jhelum found within the range of  $3.6\text{-}30 \text{ mg kg}^{-1}$  investigated by Gomez et al. (1993); Bartakova (2007) ( $0.00\text{-}15.51 \text{ mg kg}^{-1}$ ); Luis Carilous (2007) for Eucalyptus honey sample ( $17.46 \text{ mg kg}^{-1}$ ) and  $9.6\text{-}30.91 \text{ mg kg}^{-1}$  (Rural, 2008). However, the HMF content of honey samples collected from various areas of Punjab were higher than reported by Thrasyvoulou and Manikis (1995) in pine ( $2.7 \text{ mg kg}^{-1}$ ), fir ( $2.1 \text{ mg kg}^{-1}$ ), chestnut ( $4.5 \text{ mg kg}^{-1}$ ), thyme ( $5.6 \text{ mg kg}^{-1}$ ), cotton ( $5.8 \text{ mg kg}^{-1}$ ), sunflower ( $4.8 \text{ mg kg}^{-1}$ ), orange ( $5.6 \text{ mg kg}^{-1}$ ) and Erica ( $4.3 \text{ mg kg}^{-1}$ ).

### Diastase Activity (DN)

Diastase is a starch hydrolyzing enzyme in the honey. In Pakistani samples diastase activity was 28 DN in Jhang, 33 DN each in Bahawalpur and Kalar Kahar, 29 DN in Multan and 30 DN in Jhelum. The diastase activities in *A. mellifera* honey samples from Punjab, were well within the DN range recommended as quality criteria by International Honey Standard and by Directive 2001/110/EC from Council of European Union (2001), according to which DN should be 8 in healthy honey. Gomez et al. (1993) measured diastase number averaged at 20.12 DN in Spanish Eucalyptus honey and Abu-Tarboush et al. (1993) observed 6.8-37 DN in Saudian honey of different botanical sources. Thrasyvoulou and Manikis (1995) reported 23.05 DN ( $8.6\text{-}51$ ) in 8 unifloral Greek honey in 1995. Tsigouri and Passaloglou-Katrali (2000) reported average diastase number of 18.9 ( $10.3\text{-}32.4$ ) for

thyme Greek honey. Cervantes et al. (2000) found 32.5 DN and 34.17 DN, respectively, in Tahonal and Dzidzilche honey samples from Mexico. Iglesias et al. (2004) determined diastase activity in the honey samples from central Spain at an average of 29.7 DN. The comparison between the present study for DN and previous DN values reflect that diastase is a stable enzyme in honeys and shows evenness in its occurrence in them from various geographical regions and floral sources.

The physicochemical analysis of fresh Pakistani honey shows that it is of good quality because various quality evaluating parameters were found well within the permissible International standards.

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**AUTHORSHIP AND CONTRIBUTION DECLARATION**

S. No	Author Name	Contribution to the paper
1.	Dr. Samina Qamer	Conceived the idea, Methodology, Overall management of the article
2.	Ms. Irum Nasir	Data collection
3.	Ms. Um-Habiba Zafar	Data collection
4.	Dr. Salma Sultana	Data entry in SPSS and analysis
5.	Dr. Tayyaba Sultana	Data entry in SPSS and analysis

*(Received August 2015 and Accepted January 2016)*