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Comparative analysis of physicochemical properties of honey from ecological zones and branded honey of Pakistan

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Abstract

The investigations were carried out in the Honey Analysis Laboratory of Honeybee Research Institute, National Agricultural Research Centre Islamabad, Pakistan, to determine the quality and Physico-chemical characteristics of honey samples from different localities of Pakistan. Physicochemical analysis of these samples was carried out using AOAC methods and Harmonised methods of International Honey Commission and results were compared with the standards of Codex Alimentarius and European Union Council Directive. The range of different parameters was 18.00±0.10-23.50±0.30% moisture, 3.67±0.006-7.07±0.006 pH, 8.03±0.35-35.66±2.51meq/kg acidity, 6.76±0.05-7.90±0.10% sucrose, 75.00±0.20-81.00±0.10% total sugars and 0.11±0.01-0.92±0.01 mS/cm electrical conductivity, 0.01±0.003 -0.26±0.004% ash. Values for sucrose, acidity, total sugars and ash were within the normal ranges except for the moisture, pH and electrical conductivity. These parameter's values were found higher than the recommended ranges. One sample LB-19 had the highest value of 23.5±0.30% for moisture and 0.92±0.01mS/ cm for electrical conductivity, while LB-20 had the highest value of 7.07±0.006 for pH.

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Introduction

Honey is a sweet viscous fluid made mostly from the nectar of plants and is among the major bee products. Honey is defined as "the sweet substance produced by the honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs" (Codex Alimentarius, 2001). Blossom honey is derived from the nectarines of flowers, while honeydew honey is derived from the sugary excretion of some hemipterous insects on host plant or from the exudates of plants (Saxena *et al*., 2010). Honey is a concentrated invert sugars solution that contains a number of carbohydrates, aromatic substances, waxes, amino acids and organic acids, pollen grains, minerals and pigments (Bogdanov *et al*., 1998; Qiu *et al*., 1999). There are many reports on the presence of unstable compounds e.g. enzymes, vitamins and few minor compounds (Coco *et al*., 1996; Crews *et al*., 1997).

Honey is widely used in food, medicine and sweetening etc. (Sa-nguandeegul, 2003). It is produced in a lot of countries of the world and is an important source of energy. Though, honey is not a complete food according to nutritional standards but it has the potential dietary supplements. Honey is easily digestible and more palatable food than saccharose (Mendes *et al*., 1998). Due to this reason, honey is nutritionally a valuable product for athletes, children and convalescents (Erejuwa *et al*., 2012). Many scientists have worked on the physical and chemical properties of different honey types. Recently, the worldwide demand to foods that are important for human health is increasing. Honey has a preference in this regard as it also contains oligosaccharides besides various other valuable nutrients.

Honey composition and quality depends upon various factors such as, weather condition during honey production, composition of nectar, beekeeping practices and handling method during honey extraction and storage (Marchini *et al*., 2006; Iglesias *et al*., 2012).

Physicochemical characteristic of honey may also depend on the bee species as well as geographical origin. Honey has a long history of use as a medicine for the treatment of various diseases, ailments and wound (Abell *et al*., 1996 and Basualdo *et al*., 2007). Several researchers found out that the wound healing capacity of honey is greatly influenced by the physico-chemical properties of honey (Mundu *et al*., 2001; Adenekan *et al*., 2010; Nwankwo *et al*., 2014).

Honey is usually evaluated through physicochemical analysis of its constituents. These constituents are very important for honey industry as they influence the texture, flavor, granulation, storage quality and the nutritional quality of honey and have been found to be greatly important to distinguish and comparison the natural honey from artificial honey. These physicochemical parameters are recognized as criteria for the characterization and identification of honey. A thorough knowledge of these characteristics also helps in the proper packaging and storage of honey, thus, preserving its quality and taste (Clement, 2002). Therefore, International Honey Commission (IHC) has proposed certain constituents as quality criteria of honey. These constituents include: moisture content, free acidity, electrical conductivity, reducing sugars, sucrose content and minerals (Bogdanov *et al*., 1999).

One of the main factors that determine the characteristic value of honey is its floral and geographical origin (Estevinho *et al*., 2012). Therefore, Honey's composition also depends on the botanical origin, environment, climate and weather conditions, as well as handling and processing techniques (Küçük *et al*., 2007). Although major constituents of honey are almost the same in all honey samples still the chemical and physical properties of natural honeys differ depending on the floral type (Estupinon, 1998; Sa-nguandeegul, 2003; Mendes *et al*., 1998). Furthermore, variable climatic conditions are also an important factor that can also affect the honey properties.

Significant differences may exist between honeys not only due to floral types but also due to geographical differences. Most of the people don't know much about the honey that they consume. So, it is important to perform a comparative test of honey from different areas to find out the problems related to adulteration of honeys. Therefore a study was conducted to test the quality of different honey types from different localities for comparison with International Honey Standards to have a useful data for the honey consumers.

Materials and methods

The present study was conducted in the Honey analysis laboratory of Honeybee Research Institute, (NARC) Islamabad. The study was designed to compare the physicochemical properties of 20 honey samples including some local branded honey samples collected from different areas of Pakistan by Honeybee Research Institute (NARC) and following parameters were evaluated: water content (g per 100 g), pH, electrical conductivity (mS/cm), free acidity (meq/kg), reducing sugars (g per 100 g), apparent sucrose (g per 100 g) and Ash % determination.

Physicochemical parameters were analyzed using "The Official Methods of Analysis of Association of Official Analytical Chemists" (AOAC, 2002) and "The Harmonised Methods of the European Honey Commission" (Bogdanov *et al*., 1997).

Determination of Moisture Content

The moisture content of honey is the only compositional criteria, which has to be met as Honey Standard and is regulated for safety against fermentation for all the world trade honeys. It is also a useful parameter for describing honey moistness and viscosity. The moisture content of honey depends on various factors, for example, the harvesting season, the degree of maturity reached in the hive and the environmental factors (Acquarone *et al*., 2007).

According to Evangelista-Rodrigues *et al*. (2005), Apis bees operculate the combs only, when the honey moisture content ranges between 17% and 18%.

The moisture content influences some important characteristics of honey, such as viscosity and ºBrix (Anupama *et al*., 2003) The moisture content also influences the specific weight, crystallization, flavor, palatability and preservation also contributes to the development of fermenting microorganisms (Abramovic *et al*., 2008; Almeida, 2002; Araújo *et al*., 2006; Silva, 2007) According to some studies, the higher the moisture level the greater the development of microorganisms (Özcan *et al*., 2006).

In this study, Moisture content of honey samples was determined by using ATC digital Refractometer. One drop of honey was taken on the glass surface of Refractometer. The sample was covered on the surface of the prism evenly; after two minutes the reading of refractive index was recorded from the lens with the help of eye.

Determination of pH

pH of the honey samples was determined by using pH meter (Milwaukee-102). pH meter was calibrated by using standard buffers of pH 4 and 7. Each Honey sample (10g) was weighed accurately and dissolved in 75 ml distilled water. Honey solution was taken in a 250 ml beaker. The solution was stirred and pH electrode was immersed in it, when the meter got stable, readings were taken directly from the pH meter and pH was recorded. The pH values are important during honey extraction and storage as acidity influences the texture stability and shelf life of honey (Terrab *et al*., 2003). The low pH of honey inhibits the growth and presence of microorganisms and makes it compatible with many food products.

Determination of Acidity

The acidity in honey is due to the presence of organic acids, mainly gluconic acid and inorganic ions such as phosphate and chloride (Nanda *et al*., 2003). Honey is generally acidic in nature irrespective of its geographical origins. Free acidity was determined by titrimetric method. For all the samples, Honey (10g) was weighed with the help of digital balance and dissolved into 75 ml distilled water.

The solution was then titrated against 0.1 N NaOH solutions in a burette using phenolphthalein as an indicator. The titration was carried out till the solution turns to pink from colorless. The acidity was determined by using the formula. % Acidity = Vol. of NaOH used × Weight of Honey

Determination of Sugars

The main source of the sugar in honey is nectar or honeydew. It was determined by potentiometric titration using the Fehling's test (Lane and Eynon modified method). Honey (5.00g) was taken in a beaker, distilled water was added and dissolved in it to make volume up to 100 ml. 2-3 drops of phenolphthalein were added then NaOH solution was added till the solution turned to pink color. Then HCl was added to the solution till it turned to its original color and then distilled water was again added to make total volume 200ml (honey solution). Fehling solution A (5ml) + Fehling solution B (5ml) were taken in a conical flask and boiled for 2 minutes. During boiling 3 drops of Methylene blue indicator were added and then titrated with honey solution till brick red color appeared and noted as end point. The volume of honey solution used in titration was noted and following formula was used to calculate the percentage of reducing sugars in the sample.

$$\text{Reducing Sugar \%} = \frac{\text{Fehling solution constant } (0.051) \text{ x total volume of solution x } 100 /}{\text{Wt of sample solution x volume of titrate}}$$

Again (5.00g) of honey was taken in a beaker distilled water was added to bring volume up to 100 ml. 2-3drops of phenolphthalein were added then NaOH solution was added till the solution turns in to pink color. After that HCl was added to the solution till it turns to its original color and then distilled water was added again to make total volume 200 ml (v1). Took 50ml (w2) from the above solution and 50 ml distilled water was added to it to make volume up to 100 ml then 5g citric acid was added to it and solution was boiled for 10 minutes and then cooled. Then it was neutralized as in reducing sugars and distilled water was added again to make total volume up to 200 ml (v2).

Then 5ml Fehling solution A and 5ml Fehling solution B was taken and boiled for 2 min, Methylene blue 2-3 drops were added and titrated with honey solution till it turns in to brick red color. The volume of honey solution used in titration was noted and following formula was used to calculate the percentage of total sugars in the sample.

$$\text{Total sugars} = \frac{\text{Fehling solution constant } (0.051) \times 200 \times 200 \times 100}{5 \times 50 \times \text{Vol.of Honey Solution used for titration}}$$

Sucrose

The percentage of sucrose was worked out as follows:

Non-reducing sugars = total sugars – reducing sugars

Determination of Electrical Conductivity

Electrical conductivity values depend on the mineral content of honey and measures all the ionisable organic and inorganic substances. The Electrical Conductivity was determined by using conductivity meter (Milwaukee-301). Electrical Conductivity meter was first calibrated with distilled water and then the conductance cell was dipped into honey Solution (10.0%) and reading was noted in mS/cm after the stabilization of instrument. Electrical Conductivity depends on the mineral content, polyol content, protein, organic acid and complex sugars of honey and varies with its botanical origin (Terrab *et al.*, 2003).

Determination of ash

Honey (5g) was weighed accurately into a pre-weighed silica dish and was gently pre-heated on a hot plate until the sample turned black and dry so that there is no danger of loss by foaming and overflowing. The sample was then ignited at 550°c to constant weight. The sample was cooled in a dessicator before weighing. The results are expressed as % Ash and were calculated according to following formula:

$$\text{Ash \%} = \frac{W_3 - W_1}{W_2} \times 100$$

Where,

W_3= weight of dish + ash

W_1= weight of dish only

W_2= weight of honey sample

Statistical analysis

Complete randomized experimental design was used. Difference among the twenty honey samples collected from the different localities was determined by one-way Analysis of Variance (ANOVA) (Arora *et al*., 2007) using the General Linear Model (GLM) in the PC Statistical Software Statistix version 8.1. All the tests were performed in triplicates and standard deviation was calculated. Comparison between the means was done using the least significant difference (LSD) at alpha=0.05.

Results and discussion

The results of physicochemical analysis of twenty honey samples are given in the tables 1-2. All the analysis tests were done in triplicates. All of twenty samples were tested for moisture, electrical conductivity, pH, Ash, acidity, total sugars and sucrose. ANOVA was used to find out the significance difference among various variables.

Table 1. F-values (the statistic used to determine if the effects of location significantly impact the measurement from the Analysis of Variance for different physicochemical properties of honey samples.

Source of variation	DF	Moisture %	Electrical conductivity mS/cm	pH	Acidity meq/kg	Total sugars %	Sucrose %	Ash %
Location	19	118**	3506**	4630**	116**	298**	23.9**	1876**
Error	40							
Total	59							

**= highly significant at (p>0.01).

The results (Table 1) indicate significant statistical difference among different types of honey from different locations. Means were separated using least significant difference (LSD) test (table 2).

The results of all the parameters of honey samples collected from the different areas of Pakistan were compared with the European and Codex Alimentarius Standards (Codex Alimentarius, 2001; EU Council Directive, 2002). Variable means sharing same or a common letter show statistically no difference between any of the two means using LSD ($p<0.05$). Least significant difference (LSD) method compares the means for each pair of factor levels. If the difference of the two means appears greater than the LSD value than it means that they are significantly different from each other.

Acidity value of all the samples found to be well within the range of 50 milliequiv acid/kg as proposed by Codex Alimentarius (2001) and showed acidity values in the range of 8.03±0.35-35.66±2.51. Nasiruddin *et al*., (2006) reported acidity value in the range of 23.55-58.52 meq/kg for local honeys, whereas, Kamal *et al*., (2002) reported just 6.73-22.9 meq/kg of acidity in different honey samples.

The variations in acidity may be due to different nectar sources from different areas of Pakistan. As a general rule, honey irrespective of its geographical origin, is naturally acidic (Adebiyi *et al*., 2004; Khalil *et al*., 2010).

Similarly, sucrose, total sugars and ash% values were also within the standard range, as for sucrose (not more than 8 %), sum of reducing sugars (not less than 65 %) and ash % (not more than 0.6%) proposed by Codex Alimentarius and International honey commission.

Kamal *et al.,* (2002) conducted a comparative study of different floral honeys of Pakistan and reported 77 and 71% total sugar in different honey varieties. Joshi (1997) also reported total sugar in the range of 53.30 to 80.70% in different honeys. Latif *et al*., (1956) studied composition of native honey and found 65 to 76% sugar in different honey varieties.

Table 2. Means difference in physicochemical properties of honey samples from different locations.

Sample Number	Moisture %	Electrical conductivity mS/cm	pH	Acidity meq/kg	Total sugars %	Sucrose %	Ash %
L1	19.00±0.10 g	0.70±0.10 c	5.44±0.01 f	30.00±1.00 b	79.50±0.10 c	7.80±0.10 a	0.14±0.002 d
L2	19.50±0.10 f	0.56±0.006 e	6.66±0.02 b	24.66±2.51 c	78.50±0.10 e	7.86±0.05 a	0.04±0.001 i
L3	18.50±0.20 h	0.77±0.006 b	5.71±0.006 e	22.33±1.52 de	79.50±0.20 c	7.50±0.10 cd	0.17±0.001 c
L4	19.00±0.20 g	0.64±0.01 d	5.03±0.03 g	21.00±1.00 ef	79.00±0.10 d	6.76±0.05 i	0.11±0.003 e
L5	18.00±0.30 i	0.38±0.006 f	5.78±0.03 d	12.00±2.00 i	81.00±0.10 a	7.80±0.10 a	0.08±0.002 f
L6	18.96±0.05 g	0.77±0.006 b	6.62±0.02 b	8.66±1.52 j	79.00±0.10 d	7.76±0.05 ab	0.18±0.004 b
L7	19.00±0.30 g	0.24±0.006 h	3.78±0.006 m	35.66±2.51 a	80.00±0.20 b	7.80±0.20 a	0.03±0.002 j
L8	18.00±0.10 i	0.29±0.006 g	3.81±0.01 lm	21.00±1.00 ef	81.00±0.30 a	7.20±0.20 fg	0.07±0.001 g
L9	21.23±0.25 d	0.23±0.01 i	3.85±0.006 jkl	18.00±0.20 jh	77.00±0.10 g	7.16±0.05 fgh	0.03±0.004 j
L10	22.50±0.50 c	0.39±0.006 f	4.25±0.006 h	17.03±0.15 h	76.00±0.20 h	7.50±0.20 cd	0.08±0.003 f
L11	18.50±0.10 h	0.11±0.01 m	3.78±0.01 m	12.36±0.35 h	79.00±0.10 d	7.40±0.10 de	0.02±0.002 k
L12	19.50±0.50 f	0.24±0.01 hi	3.9±0.010 j	18.00±0.30 gh	78.50±0.30 e	7.60±0.10 bc	0.08±0.004 f
L13	21.00±0.50 d	0.14±0.01 kl	3.87±0.006 jk	24.33±2.08 cd	78.50±0.10 e	7.26±0.05 ef	0.05±0.005 h
LB14	20.00±0.20 e	0.16±0.006 j	3.67±0.006 n	35.03±0.25 a	78.46±0.15 e	7.20±0.10 fg	0.03±0.001 j
LB15	23.00±0.10 b	0.15±0.01 k	3.83±0.02 klm	20.03±0.15 fg	75.50±0.10 i	7.16±0.05 fgh	0.02±0.004 k
LB16	19.00±0.10 g	0.13±0.01 l	3.81±0.01 lm	24.96±0.25 c	79.50±0.20 c	7.00±0.10 h	0.01±0.004 l
LB17	18.96±0.15 g	0.24±0.006 h	3.86±0.01 jkl	21.03±0.25 ef	79.50±0.10 c	7.30±0.10 ef	0.08±0.005 f
LB18	20.00±0.10 e	0.11±0.01 m	4.14±0.12 i	20.56±1.25 ef	78.00±0.10 f	7.20±0.20 fg	0.01±0.003 l
LB19	23.50±0.30 a	0.92±0.01 a	6.52±0.006 c	8.96±0.15 j	75.00±0.20 j	7.90±0.10 a	0.26±0.004 a
LB20	19.50±0.10 f	0.90±0.006 a	7.07±0.006 a	8.03±0.35 j	78.50±0.10 e	7.06±0.05 gh	0.25±0.003 a
LSD(p<0.05)	0.4223	0.0136	0.0506	2.0501	0.2669	0.1929	4.969

Values of Ash % are similar to those of Ihtisham-Ul-haq (1997) who determined ash content of different types of honey and draw an ash % range of 0.008 to 0.49% in honey samples. These results are also in accordance with those of White (1975) who obtained ash content of different varieties of honey in the range of 0.020 to 1.028%. The variation may be due to many factors such as soil composition, atmospheric conditions and floral type. The mineral content of honey differ according to the plant species that honey bees visit during nectar collection, and also the soil type in which the plant is located. These contents play a major role in biochemical and physiological functions of human body and some are essential for the growth and development of bones and muscles (Turan *et al*., 2003).

All the tested honeys except five samples showed moisture below 20%, which is the maximum limit for moisture content according to Codex honey standard (Codex Alimentarius, 2001). In the Codex Alimentarius Standards (2001) and EU Council directives (2002), the maximum moisture value of pure floral honey is generally less than 20%.

Moisture of five honey samples L9, L10, L13, L15 and L19 was much higher than the maximum moisture limit of 20%. Two samples L5 and L8 had lowest value of moisture i.e 18.0%, which is significantly quite different from the L3, L6, L11 and L17 that showed moisture means of 18.50%, 18.96%, 18.50% and 18.96% respectively. L19 had the highest of moisture value of 23.50% that is significantly highly differently from all the other samples.

According to moisture % the honey samples can be catagorized into three groups. One group had moisture in the range of 18.0-19.5% the other have 20.0-22.5% moisture. One sample L15 had 23.0% moisture, while L19 showed highest moisture of 23.5%. Fredes and Montenegro (2006) concluded that honeys having low moisture content will exhibit a better shelf life. Usually ripe honey has moisture content below 18.6% (Bogdanov *et al*., 1999). Joshi *et al*., 2000 found out that moisture content in *A. dorsata* honey was higher (21.5%) than that of *A. mellifera* (17.1%).

National beekeeping organizations of some countries (e.g. Germany, Belgium, Austria, Italy and Switzerland) have set a maximum range of 18%–18.5%, while the European Union has set a maximum value of 20% moisture content (Codex Alimentarius Commission, 2001).

Mostly the samples had pH in the normal range of 3.0-6.4. Three samples L2, L6 and L19 have pH 6.66, 6.62 and 6.52 respectively. While one sample showed highest pH value of 7.07. All of them are significantly different from each other (statistical significance is mainly determined by the p- value of a hypothesis test. If the p-value is low, then the statistic is considered to be statistically significant). The pH (means) of eight samples that is L7, L8, L9, L11, L12, L13, L15, L16 and L17 are 3.78, 3.81, 3.85, 3.78, 3.90, 3.87, 3.83, 3.81 and 3.86 respectively. They do not show statistically significant differences. Ten samples L1, L2, L3, L4, L5, L6, L10, L14, L18 and L19 have pH means of 5.44, 6.66, 5.71, 5.03, 5.78, 6.62, 4.25, 3.76, 4.14 and 6.52 respectively. Which are significantly very different from the above eight samples. While one sample L20 had a significantly highly different pH as compared to others (7.07). The difference in pH values can be due to the fact that the growing season and geographical locations affect the percentage of different elements in honey. These results are also in accordance with those of Hussain (1989) who reported pH in the range of 3.0 to 5.0 for pure honey. Honeys with a pH range of 3.5 to 4.5 are considered to have originated from nectar (Amir *et al*., 2010).

Table 3. Geographical origins of honey samples (N = 20) used in this study.

Sample code	Geographical origin
L1	Chakwal
L2	Karak
L3	Mianwali
L4	Bannu
L5	Peshawar
L6	Fateh Jhang
L7	Toba Tek Singh
L8	Chitral
L9	Margalla
L10	Satra mile, Islamabad
L11	Dina, Jehlum
L12	Haripur
L13	Peshawar
LB14	Branded
LB15	Branded
LB16	Branded
LB17	Branded
LB18	Branded
LB19	Branded
LB20	Branded

The electrical conductivity (EC) values of most of the honey samples were in the range of 0.1-0.80 mS/cm. Two samples L19 and L20 had highest electrical conductivity of 0.92 and 0.90, which is more than the normal range (0.8mS/cm).

The electrical conductivity is a good indicator of the honey's botanical origin. Its measurement depends upon the acid and ash and content of the honey, so higher the acid and ash content, higher will be the conductivity.

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References

Abell DC, Frube H, Schweger C, Kwok ASK, Sporns P. 1996. Comparison of processed unifloral clover and canola honey. Apidologie **27**, 451-480.

Acquarone CP, Buera, Elizalde B. 2007. Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food Chemistry **101,** 695–703.

Adebiyi FM, Akpan I, Obiajunwa EI, Olaniyi HH. 2004. Chemical/physical characterization of Nigerian honey. Pakistan Journal of Nutrition **3(5),** 278-281.

Adenekan MO, Amusa NA, Okpezel VE, Owosibo AO. 2012. Nutritional and Microbiological Composition of Honey Samples obtained from Ogun State, Southwestern Nigeria. European Journal of Sustainable Development **1(2),** 271-286.

AOAC. 2002. The Official Methods of Analysis of Association of Official Analytical Chemists, 17th Ed (edited by W. Horowitz). pp. 22-33. Gaithersburg, MD: AOAC International.

Amir Y, Yesli A, Bengana M, Sadoudi R, Amrouche T. 2010. Physico-chemical and microbiological assessment of honey from Algeria. Elec. J. Envi., Agr. and Food Chem. **9,** 1485 – 1494.

Arora PN, Sumeet Arora Arora S. 2007. Comprehensive Statistical Methods. Chapter 19, pp **19(1),** 19-37.

Basauldo C, Sgroy V, Finola MS, Marioli MJ. 2007. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Veterinary Microbiol. **124,** 375-381.

Bogdanov S, Martin P, Lullmann C. 1997. Harmonised methods of the European Honey Commission. Apidologie. **28,** 1-59.

Bogdanov S, Kilchenmann V, Fluri P. 1998. Influence of organic acids and components of essential oils on honey tastes. Am. Bee. J. **139,** 61-63.

Bogdnov S, Martin P, Lullman C, Borneck R, Von der ohe W, Russmann R. 1999. Honey Quality, methods of analysis and international regulatory standards: review of the work of the International Honey Commision. Bee World. **80,** 61-69.

Clement H, Bruneau E, Barbancon JM, Bonnaaffe J, Reeb C, Veissiere B. 2002. Le traite rustica de l'apiculture. Paris; Traite Rustica 528 p.

Coco FL, Valentini C, Sapra V, Ceccon L. 1996. High performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in honey. J. of Chromatography A., **749,** 95-102.

Codex Alimentarius Commission. 2001. Revised codex standard for honey. Codex Standard 12-1981. Rome: FAO and WHO food standards programme.

Crews C, Startin JR, Clarke PA. 1997. Determination of pyrrolizidine alkaloids in Honey from selected sites by solid phase extraction and HPLC-MS. Food Addition Contamination., **14,** 419-428.

Erejuwa OO, Sulaiman SS, Wahab MSA. 2012. Honey: A novel antioxidant. Molecules. **17,** 4400–4423.

Erejuwa OO, Sulaiman SS, Wahab MSA. 2012. Fructose might contribute to the hypoglycemic effect of honey. Molecules. **17,** 1900–1915.

Estevinho LM, Feás X, Seijas JA, Vázquez-Tato MP. 2012. Organic honey from Trás-Os-Montes region (Portugal): chemical, palynological, microbiological and bioactive compounds characterization. Food Chem. Toxicol., **50(2),** 258–264.

Estupinon S, Sanjuan E. 1998. Quality parameters of honey II chemical composition. Alimentaria.. **297**, 117-122.

EU. 2002. Council Directive 2001/110/CE relating to honey. Official Journal of the European Communities **10,** 47–52.

Evangelista-Rodrigues A, Silva SEM, Beserra EMF, Rodrigues ML. 2005. Análise físico-química dos méis das abelhas Apis mellifera e Melipona scutellaris produzidos em duas regiões no Estado da Paraíba. Cienc Rural. **35,** 1166-1171.

Fredes C, Montenegro G. 2006. Heavy metal and other trace elements contents in honey bee in Chile. Cien. Inv. Agric., **33,** 50-58.

Hussain MH. 1989. Studies on the production and some properties of honey's from Dhofer (Oman). Proc. Fourth Intl. Conf. Api. Tropical Climates. Int. Bee Res. Asso. 256-264.

Iglesias A, Feás X, Rodrigues S, Seijas JA, Vázquez-Tato MP, Dias LG, Estevinho LM. 2012. Comprehensive study of honey with protected denomination of origin and contribution to the enhancement of legal specifications. Molecules **17,** 8561–8577.

Ihtisham-ul-haq. 1997. Effect of floral type, geographical locations and honey bee species on the water activity and other physico-chemical parameters of honey. M.Sc. Thesis. Agricultural University, Peshawar, Pakistan.

Joshi VK. 1997. Fruit Wine, Nauni, Solan (HP). Directorate of Extension Education, Uni. of Hortic. and Forestry, Ch. 2.

Joshi SR, Pechhacker H, William A, Von Der Ohe W. 2000. Physico-chemical characteristics of *Apis dorsata*, *A. cerana* and *A. mellifera* honey from Chitwan district, central Nepal. Apidologie, **31,** 367–375.

Kamal A, Raza A, Rashid N, Hammed TG, Lami M, Gureshin MA, Nasim K. 2002. Comparative study of Honey collected from flora of Pakistan. Online J. Biol. Sci. **23(9),** 626-627.

Khalil MI, Sulaiman SA, Boukraa L. 2010. Antioxidant properties of honey and its role in preventing health disorder. The open Nutraceuticals Journal **3(1),** 6-16.

Küçük M, Kolailı S, Karaoğlu S,. Ulusoy E, Baltacı C, Candan F. 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem., **100**, 526–534.

Latif A, Qayyum HA, Haq M. 1956. Research on the composition of native honey. Pak. J. Sci. Res., **8**, 163-165.

Mendes E, Brojo Proenc E, IMPLVO, Ferreira, Ferreira MA. 1998. Quality evaluation of Portuguese honey. Carbohydrate Polymers. **37,** 219-223.

Marchini LC Reis VDA, Moreti ACCC. 2006. Composição físico-química de amostras de pólen coletado por abelhas africanizadas *Apis mellifera* (Hymenoptera: Apidae) em Piracicaba, Estado de São Paulo. Ciência Rural, **36(3),** 949-953.

Maundu MA, Padilla-Zakour OI, Worobo RW. 2001. Antimicrobial activity of honey against food pathogens and food spoilage microorganisms.Vol.1.W.North St. Geneva, N.V 14456; Department of food sciences and technology, Cornell University, NYSAES. 61-71.

Nanda V, Sarkara BC, Sharma HK, Bawa ASV. 2003. Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. J. Food Composit. Anal., **16,** 613-619.

Nasiruddin UK, Qaiser M, Raza SM, Rehman M. (2006). Physicochemical properties and pollen spectrum of imported and local samples of blossom honey from the Pakistani market. Int. J. Food Sci. Technol., **41,** 775-781.

Nwankwo CM, Ezekoye CC, Igbokwe SO. 2014. Phytochemical screening and antimicrobial activity of apiary honey produced by honey bee (*Apis mellifera*) on clinical strains of *Staphylcoccus aureus, Escherichia coli and Candida albicans*. African Journal of Biotechnology, **13(23),** 2367-2372.

Pe´rez-Arquillue C, Conchello P, Arino A, Juan T, Herrera A. 1994. Quality evaluation of Spanish rosemary (*Rosmarinus officinalis*) honey. Food Chemistry **51,** 207–210.

Qiu PY, HB Ding YK, Tang RJ Xu. 1999. Determination of chemical composition of commercial honey by near infrared spectroscopy. J. of Agri. Food Chem., **47,** 2760-2765.

Sa-nguandeegul R. 2003.Honey and bee products in Thailand. Symposium on Biodiversity of Honey Bees and Bee Products, Chiang Mai, Thailand. 24-25.

Saxena S, Gautam S, Sharma A. 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. **118,** 391–397.

Terrab A, González AG, Díez MJ, Heredia FJ. 2003. Mineral content and electrical conductivity of the honeys produced in Northwest Morocco and their contribution to the characterisation of unifloral honeys. J. of the Sci. of Food and Agric., **83(7),** 637-643.

Turan M, Kordis S, Zeyin H, Dursan A, Sezen Y. 2003. Macro and micro minerals content in some wild edible leaves consumes in eastern Anatolia. Tailors and Francis. 129-130.

White JW. 1975. "Composition of Honey" in: Honey A Comprehensive Survey (Ed. Crane E); Heinemann, London. 157-206.