

Physicochemical and Microscopic Evaluation of Adulterated Honeys

Kashif Nawaz¹, Fawad Ahmad², Umair Khan³, Raees Ahmad³, Atif Mehmood⁴, Muhammad Kashif³, Asghar Hussain², Noor ul Wahab⁵, Tariq Ali Shah³, Muhammad Abbas¹, Syed Umair Shah², Firdos Khan⁶.

¹Department of Biochemistry, Abdul Wali Khan University Mardan, Pakistan.

²Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan.

³Department of Food Science and Technology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan.

⁴Department of Human Nutrition, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan.

⁵Department of Horticulture, Abdul Wali Khan University Mardan, Pakistan.

⁶Department of Horticulture, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, 25130, Pakistan.

Correspondence Author: Firdos Khan*

ABSTRACT

The current study reveals the collection of nectar from the plants, the honey bee brought it to the honey. Honey is a good source of diet which is consumed all over the world. For the comparison of physicochemical properties of honey, the research was conducted at the honey analysis laboratory of the food science research institute at NARC Islamabad. For that purpose, the five different samples of honey are brought from a local market. The basic parameters of honey which was thoroughly lookup during Research are P^H, the content of moisture, electrical conductivity, free acidity, and 5-hydroxymethylfurfural (HMF). During the research, we find out that moisture content is not only an indicator of honey purity but also a source of quality if in the desired range. Also by comparing our result of different aspect with comparing to national and international standards we observed that electrical conductivity, Ash content, and 5-hydroxymethylfurfural (HMF) is also good parameters to find out the quality and purity of honey.

I. INTRODUCTION

Approximately 80% of the honey we consume comes from beehives. Honey is produced from nectar (and flowers, the secretions of plants or from the excretions of insects) that feed on plants. Bees collect nectar, transform it by combining it with substances of their own, deposit, dehydrate, store, and allow it to mature and ripen in honeycomb (Vanhanen *et al.*, 2011). Honey is made by bee's nectar which was collected from the flowers, sap from plants, and some proportion of honeydew were utilized. Honey's color, aroma, and consistency are all determined by the flowers foraged by the bees. Female worker bees are always forager honeybees. Drone bees and queen bees never go foraging for food. Honey's flavor and composition are affected based on a number of factors, such as the source of the nectar(s) from which it is made and to a lesser extent, external influences such as climatic conditions and beekeeping procedures for removing and extracting honey (Luis *et al.*, 2009). The Honey bees choose their foraging plant's mostly based on the amount of sugar in the plant's nectar, which is the honey's basic component (Crane, 1980). The Pollen grains are most commonly found in flowery nectar and are consequently the major source of pollen grain in honey. Furthermore, there are a variety of ways to deliver external pollen into a beehive. The pollen grains can come from bee's body portions into nectar-filled combs, air-borne pollen grain can enter the store through air currents, discarded wax combs can be introduced to hives, or imported pollen grain can be given to bee's (Anonymous, 2008). Honey is frequently consumed as the main source of energy. Honey works well in both hot and cold drinks as a sweetener. It goes well with almost every meal. Honey's moisture-absorbing properties help bread, cakes, biscuits, and candies last longer (MAAREC, 2004). Honey is a concentrated water mixture of two sugars, dextrose, and laevulose, plus fewer quantities of at least twenty-two other than extra complex sugars. Flavoring compounds, pigments, organic acids, and minerals are all minor ingredients in honey. Fructose, glucose, and water are the three principal components of floral honey, accounting for 38.2 percent, 31.3 percent, and 17.2 percent, respectively, according to surveys. Honey contains only glucose and fructose as monosaccharides. The di and trisaccharide fractions of floral honey are made up of these sugars in various ways. Sugar makes up 95 to 99 percent of the dry matter in honey and 85 to 95 percent of the total sugars. Honey's sweetness, hygroscopic characteristics, energy value, and other physical features are all due to these simple sugars (Buba *et al.*, 2013). Bee honey's properties and composition are

influenced by its geographical floral origin, season, ambient variables, and beekeeper care (EL-Metwally, 2015). Bee honey is one of the few meals that the body can readily digest and is virtually allergy-free. It contains nutrients that are particularly useful as an energy source (Rahman et al., 2010). Pollen grains are always present in natural honey that has been treated according to regulated procedures. The floral diversity and species composition of the plants foraged by honey bees, and thus the melliferous plant species available in the vicinity of the apiary, are reflected in the pollen content of the honey, which not only reflects regional agricultural practices and forest vegetation, but also the floral diversity and species composition of the plants foraged by honey bees and, as a result, the melliferous-plant species available in the vicinity of the apiary (Louveaux *et al.*, 1978). It is a wonderful source of energy and is high in micronutrients. For generations, it has been used as a medicine to cure a variety of diseases. Consider the following scenario: Sore throats, cuts, and burns can all benefit from honey. Pollen can be utilized to slow down the aging process and, when coupled with honey, is a nutritious diet for sick individuals. (Lay-flurrie, 2008).

ADULTERATED HONEY

The complex methods of adulteration are continually being introduced and the authorized assessment of honey quality parameters is in-adequate to identify maximum types of honey adulteration. Honey adulteration is a hot topic. Furthermore, while honey's appeal among consumers continues to expand, its global production is insecure. Honey is one of humanity's earliest foods. It contains a large amount of nutrient-containing compounds which promote good health and recovery. It's a sweet product by definition. Honey is becoming a rare product.

HONEY ADULTERATION

Honey adulteration is either direct or indirect.

- The directly introducing of a substance to honey is known as direct adulteration.
- When honeybees are fed an adulterating chemical, this is known as indirect adulteration.

Honey adulteration by indirect means:

Honey is indirectly adulterated by feeding synthetic sugars to honeybees at the moment when broods become naturally available. It's incredibly difficult to detect such indirect adulteration.

Honey's Chemical Composition

Carbohydrates unsurprisingly, make up the majority of honey, accounting for approximately 82 percent. The mono-saccharides fructose (thirty-two percent) and glucose (thirty-one percent) are present, as well as the di-saccharides such as maltose, iso-maltose, maltulose sucrose, turanose, and kojibiose (nine percent). Some of the oligo-saccharides (four percent), such as panose, thean-derose, and, erlose, are generated when the higher saccharides in nectar and honeydew are not completely broken down.

AMINO ACIDS AND PROTEINS

Invertase enzyme breaks down sucrose to fructose and glucose. The amylase enzyme breaks down starch into small units. The glucose oxidase enzyme breaks down glucose to gluconolactone which yields gluconic acid and hydrogen peroxide. The catalase enzyme breaks down the per-oxide produced by the glucose oxidase enzyme to water (H₂O) and oxygen (O₂). The phosphorylase removes inorganic phosphate from organic phosphates. The honey also includes 18 free amino acids, with proline being the most prevalent.

MINERALS, VITAMINS, AND ANTI-OXIDANTS

The folic acid, niacin, riboflavin, panto-thenic acid, and vitamin B-6 are all found in trace amounts in honey. It also contains zinc, iron, calcium, potassium, magnesium, selenium, chromium, phosphorous, manganese, and also contains minerals. The flavonoids are the principal antioxidants in honey, and one of them, pinocembrin, is only found in honey and bee's profiles. The antioxidants include such as ascorbic-acid, catalase enzyme, and selenium. Generally dark honey acts as anti-oxidizing effect mostly.

OTHER COMPOUNDS

The honey also contains a large number of aromatic acids as well as organic acids for example acetic acid, butanoic acid, citric acid, lactic acid, formic acid, malic acid, succinic acid, pyroglutamic acid, and gluconic acids. The basic present acid is gluconic and is generated when the glucose oxidase enzyme breaks down glucose. The honey contains hydroxyl-methyl-furfural which converts sugar at less than pH 5.

- Fructose: 38.2 percent
- Glucose: 31%
- Sucrose at a rate of 1.5 percent
- Water/ moisture 17.1 %
- Minerals, vitamins, enzymes 0.5 %

- Maltose 7.2 %
- Trisaccharide carbohydrates 4.2 %

Honey Standards:

- PSQCA honey standard
- Codex Alimentarius Standard
- European Standard

1.1.1 PAKISTAN STANDARDS

The Standards Development Centre was adopted by Pakistan Standards and Quality Control Authority (PSQCA) on January 19, 2012, after the Sugar Industries Technical Committee's draught was authorized by the National Standard Committee for Agriculture and Food Products (NSCAFP). In 1982 the National Standard Committee for Agriculture and Food Products (NSCAFP) was established and it was first amended in 1992, and then again in 2007. The committee felt it was necessary to update in view of recent industry developments. All honey sold in Pakistan is divided into three types, namely: -

- The comb of honey is sold in complete combs or segments of combs which is already sealed.
- Extracted Honey is made from the combs of un-caped bees.
- Pressed Honey the rock bee *Apis darsota* provides the majority of the Pressed Honey.

Honey Standards:

Physicochemical parameters	PSQCA standards range	Codex Standards range	European Standards range
Moisture	Not more than 21%	Not more than 20%	Not more than 20%
Reducing Sugar	Not less than 65%	Not less than 60%	Not less than 60%
Sucrose	Not more than 5%	Not more than 5%	Not more than 5%
pH	3.0-6.40	3.0-6.40	3.0-6.40
Acidity	Not more than 40 meq/kg	Not more than 40 meq/kg	Not more than 40 meq/kg

Electrical Conductivity	Not more than 0.8 mS/cm	Not more than 0.8 mS/cm	Not more than 0.8 mS/cm
HMF	Not more than 40mg/kg	Not more than 40mg/kg	Not more than 40mg/kg
Diastase(after blending & processing)	Not less than 3 schade units	Not less than 3 schade units	Not less than 3 schade units
Invertase	Not more than 10	Not more than 10	Not more than 10
Total Proteins	0.2%-0.4%	0.2%-0.4%	0.2%-0.4%
Ash	Not more than 0.6%	Not more than 0.6%	Not more than 0.6%

II. MATERIALS AND METHODS

The research was conducted in the analysis laboratory of Honey at Food Science Research Institute, (NARC) Islamabad. The study was arranged to compare physicochemical properties of 05 honey samples provided by the Honey analysis laboratory, Food Science Research Institute (NARC) Islamabad, and the following parameters were assessed: moisture content (g/100 g), pH, electrical conductivity (mS/cm), 5-hydroxymethylfurfural, free acidity (meq/kg) and Ash % determination. Physicochemical parameters were evaluated using “The European Honey Commission Harmonized Methods” (Bogdanov *et al.*, 1997).

III. RESULTS AND DISCUSSION

Table I-4 reveals the results of physicochemical analysis of 05 samples of honey compared with that of International Honey Standards. All five samples were tested for moisture, acidity, pH, ash, HMF, and electrical conductivity.

According to the Codex *et al.* (2001) honey standards and the EU honey directive 2002, the moisture content is not more than twenty percent (20%). That parameter has to do with the process of fermentation which is necessary for honey preservation and its shelf life. The moisture percentage of most honey samples in our investigation ranged from 17.0-17.9% according to the results reported for Pakistani honey. Climate conditions, harvest season, honey maturation level,

and also environmental and geographical factors, all influence the moisture content of honey (Kadri *et al.* 2016). Because honey's content of water may be artificially altered during processing (Bogdanov *et al.* 2004). Hence, it is not considered a good predictor of the floral origin of honey. Most microbes grow in a pH range of 7.2 to 7.4, the pH indicates the possibility of microbial development. As a result, honey samples with pH values ranging from 3.2 to 4.5 are regarded acceptable (da Silva *et al.*, 2016). In terms of honey pH, all of the honey samples are acidic, with a pH range of 3.5 to 5.5 (Bogdanov *et al.* 2004). Despite differences in acidity, there were no differences in pH between the examined honey types (Alqarni *et al.*, 2016). Because of the buffering effects of different acids and the presence of minerals, it was discovered that these two parameters are not directly related to one another. In particular, the existence of organic acids in equilibrium with their corresponding lactones, internal esters, and some inorganic ions, such as phosphates, sulphates, and chlorides, determines how acidic the honey is (Alvarez-Suarez *et al.*, 2010). Our findings are in agreement with the pH range (3.0-6.4) described by Lazarevi *et al.* (2012).

The samples had pH in the normal range of 5.76-6.4 except sample no. 5 (6.52) which had a pH value higher than standards. The difference in values of pH can be due to the growing season and geographical origins which have an impact on the percentage of different elements in honey. These findings concur with those made by Hussain (1989), who noted that pure honey had a pH between 3.0 and 5.0. Honey with a pH range of 3.5 to 4.5 is said to be originated from nectar (Amir *et al.*, 2010).

The Values of free acidity, in the studied samples, ranged from 11.0 to 16.0 (meq kg⁻¹), which were within the range (below 50 meq kg⁻¹) according to the EU honey directive 2002, showing the absence of unwanted fermentation. It is commonly known that glucose and fructose are fermented to produce carbon dioxide and ethanol. Acetic acid (CH₃COOH), which contributes to honey's free acidity level, is produced when alcohol is further oxidized in the presence of oxygen (Moussa *et al.* 2012).

Regarding the electrical conductivity, since honey contains electrolytes, in the form of minerals and acids, samples exhibit varying grades of electrical conductivity (EC) (International Honey Commission 2009) where the higher the electrolytes content, the higher the resulting conductivity. This parameter is a very useful quality parameter for the classification of monofloral honey and can be measured by relatively low-cost instrumentation (Bogdanov *et al.* 2004). The

samples showed high values for electrical conductivity which ranges from 1.116 to 1.214 mS cm⁻¹, which was not within the range (i.e less than 0.8 mS cm⁻¹).

As previously mentioned, the mineral content of honey is strongly correlated with its ash level and can be affected by the same set of factors that affect all honey constituents. Its levels fall between 0.02 to 0.3 percent, according to Buelga et al. (2017). However in our study the ash% value range from 0.3224 and 0.6167%. Ash% values of all the samples were within the range except for one sample (Sidr-5). The permitted limit for Ash% as set by Codex et al. (2001) and the EU honey directive (2002) is not more than 0.6%.

HMF test was conducted for 5 honey samples provided by Honey Analysis Laboratory of Food Science Research Institute (NARC) Islamabad. The range of HMF in studied samples was from 37.57 to 140.11 mg/kg. The Codex Alimentarius (2001) and EU honey directive (2002) specify a maximum HMF value of 40.00 mg/kg for processed honey, and a maximum value of 80.00 mg kg/kg for honey if it is blended with a declared origin from tropical climate regions. HMF levels of one sample (H-31=140.11) were higher than the permitted range (max. 80 mg/kg for tropical climate), so the other 4 honey samples can be regarded as fresh honey. The HMF results obtained were, on average, greater than those reported in the literature for Spanish honey (6.80 mg/kg), (Terrab et al., 2002), from Turkey (2.52 mg/kg), (Turhan et al., 2007) and from Argentina (8.98 mg/kg), (Cantarelli et al., 2008). When honey is heated or stored for a long time, it decomposes into HMF (da Silva *et al.*, 2016). HMF can be created by heating sugars in the presence of an acid to the inversion of sucrose, high HMF level in honey also indicates a falsification by adding inverted syrup (Yücel & Sultanoglu, 2013).

Table-1: Pollen analysis of adulterated honey samples.

Sample	Pollen density	Primary pollen	Secondary pollen	Minor pollen	Sugar crystals density	Flora types
Sample-1	Medium	Wild Flower	Acacia	Eucalyptus, Sider	Medium	2-3

Sample-2	Low	Eucalyptus	Wild flower	Acacia, Sider	Medium	2-3
Sample-3	Medium	Sider	Acacia, Eucalyptus	Brassica, Sheesham	Medium	3-4
Sample-4	Medium	Acacia	Onion,	Eucalyptus Sider	Medium	2-3
Sample-5	Medium	Eucalyptus	Acacia	Wild flower, Sider	Medium	2-3
Sample	Pollen density	Primary pollen	Secondary pollen	Minor pollen	Sugar crystals density	Flora types

Table-3: Physicochemical analysis of adulterated honey samples

Honey Sample	Moisture % Not more than 21%	E.C (mS/cm) Not more than 0.8mS/cm	pH 3.0 – 6.40	Acidity (meq/kg) Not more than 40meq/kg	Ash % Not more than 0.6%
Wild Flower	17.2%	1.192*	6.20	11.0	1.112*
Eucalyptus	17.0%	1.116*	6.39	13.0	1.036*
Sider	17.1%	1.191*	6.13	16.0	1.111*
Acacia	17.9%	1.158*	5.76	13.0	1.078*
Eucalyptus	17.3%	1.214*	6.52*	12.0	1.134*

Table-4: HMF values of honey samples

Honey Sample	HMF (mg/kg)
Sample 1	67.81mg/kg*
Sample 2	60.47mg/kg*

Sample 3	77.02mg/kg*
Sample 4	37.57mg/kg
Sample 5	140.11mg/kg*
Standards Range	PSQCA(not more than 80mg/kg) Codex/European(not more than 40mg/kg)

Table-5 Fiehe's Test (Qualitative HMF+ Adulteration):

Serial No.	Sample Type	Fiehe's Test Result
1.	S1	Positive
2.	S2	Positive
3.	S3	Negative
4.	S4	Positive
5.	S5	Positive

CONCLUSION

The results showed that all the samples had Ash, Electrical conductivity, and HMF values out of national and international standard ranges (Ash from 1.036 to 1.134 and EC from 1.116 to 1.214). pH was high for the Sider honey sample. This suggests that Electrical conductivity, Ash%, and HMF are good parameters to assess the purity and quality of honey. It also concludes that Moisture is not a suitable parameter to check honey purity but it is very important concerning honey quality. This deviation from national and international standards with the parameters like HMF, EC, and Ash parameters thus suggests adulteration which renders the honey unsatisfactory in terms of quality and purity.

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AUTHORS

First Author – Kashif Nawaz, B.Sc (Hons), Department of Biochemistry, Abdul Wali Khan University Mardan, Pakistan

Second Author – Fawad Ahmad, M.Sc (Hons), Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan

Third Author – Umair Khan, M.Sc (Hons), Department of Food Science and Technology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Fourth Author – Raees Ahmad, M.Sc (Hons), Department of Food Science and Technology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Fifth Author – Atif Mehmood, M.Sc (Hons), Department of Human Nutrition, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Sixth Author – Muhammad Kashif, M.Sc (Hons), Department of Food Science and Technology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Seventh Author – Asghar Hussain, B.Sc (Hons), Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan

Eighth Author – Noor ul Wahab, M.Sc (Hons), Department of Horticulture, Abdul Wali Khan University Mardan, Pakistan

Ninth Author – Tariq Ali Shah, M.Sc (Hons), Department of Food Science and Technology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Tenth Author – Muhammad Abbas, B.Sc (Hons), Department of Biochemistry, Abdul Wali Khan University Mardan, Pakistan

Eleventh Author – Syed Umair Shah, B.Sc (Hons), Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan

Twelfth Author – Firdos Khan, M.Sc (Hons), Department of Horticulture, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

Correspondence Author – Firdos Khan*