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Evaluation of Physio-chemical properties of honey collected from local markets of Lahore, Pakistan

Aamir Sharif[1], Muhammad Iftikhar[2*], Ashraf Hussain[3], Mati Ur Rehman[4], Syed Faisal Zaidi[5], Muhammad Akram[6], Muhammad Daniyal[7], Khan Usman ghani[8]

Abstract: Determination of standard criteria for food products is very essential, as their validity, quality and consumption depend upon it. Honey is widely usedin food, sweetening and in medicine. The honey quality is a significant factor for both international and national (local) markets to ensure human health and to enable attainment of competitive premium prices. In developing countries, quality consideration aspect of honey is disregarded by producers and processors. The current study was conducted to determine the quality of local and imported brands of honey available in the markets of Lahore, Punjab (Pakistan) and compared with national and international standards. The study was conducted at research and development lab of Islamic Shahed Centre (Pvt) Ltd. Lahore. The tested samples were evaluated for pH, acidity, electrical conductivity, moisture, reducing sugar, sucrose, mineral (Ash), Fiehe's test and Hydroxy methyl furfural (HMF). The ranges of different parameters are 3.09-5.84 pH, 14.02-33.4 meq/kg acidity, 0.1-0.6 mS/cm electrical conductivity, 14.5-25.7 % moisture, 66.2-80.1 % total sugar, 1.33 to 14.2 % sucrose, 0.002-0.234 % ash (mineral) and 14.5 to 112 mg/kg HMF. Some of the samples showed high amount of Sucrose, HMF and moisture content and positive Fiehe's Test which confirm low quality of the samples. Generally, the findings showed that attributes of 10 (55.5%) out of 18 of the commercial honeys meet with national and international standards which make them good for human consumption while others have higher values than national and international parameters, thus suggesting adulteration and render the honey unhealthy for children and adults.

Keywords: Honey, Physico-chemical, Standard, Lahore, Adulteration

1. Research and Development Officer, Islamic Shahed Center (Pvt.) Ltd.,Lahore Pakistan
2. Research and Development Chemist, Islamic Shahed Center (Pvt.) Ltd., Lahore Pakistan
3. University of Central Punjab Lahore
4. Department of Radiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan
5. Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdul Aziz, University of Health Sciences, Jeddah, Saudi Arabia
6. Department of Eastern Medicine, Government College University Faisalabad
7. TCM and Ethnomedicine Innovation & Development International Laboratory, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208,China
8. Herbion Pakistan (Pvt.) Ltd., Karachi, Pakistan

***Corresponding author Email:** iftikharmehar67@gmail.com

INTRODUCTION

Honey, one of the major bee products, is a semiliquid, sweet and flavored food stuff produced from nectar of nectarines of flowers, or sweetjuice or excretion of plant-sucking insect onthe living parts of plant, which the bees collect, transform by addition of specific substances of their own, deposit, dehydrate, store and leave in honeycomb to ripen and mature (1, 2). Honey mainly consists of water and sugars, especially glucose and fructose (95 – 99 % of honey drymatter) and fructo-oligosaccharides (4-5% of honey dry mater). Other sugars are present in the form of disaccharides like sucrose, maltose, isomaltose, maltotroise, melezitose, meli-bose, nigerose, turanose and panose. Additional chemical substances present in the honey are minerals, enzymes, vitamins, amino acids, trace elements, nitrogenous compounds, phenol, flavonoids and antibiotic rich inhibine. (3-9). Honey is widely used in food, sweetening and in medicine (10). The honey quality is main factor for both international and national (local) markets to ensure human health and to enable attainment of competitive premium prices (11). In developing countries, quality consideration aspect of honey is disregarded by producers and processors. The major cause of quality deterioration includes high moisture content, heating at high temperature, adulterations, poor storage conditions and packaging (11). Honey is usually assessed byphysiochemical analysis of its components. Many of these components affect texture, granulation, flavor, nutritional quality, and storage quality of honey and have a great significance in honey industry. They are also responsible for the medicinal quality of honey. Certain constituents are therefore proposed as quality criteria for

honey by International Honey Commission (IHC). These constituents are sucrose content, reducing sugars, electrical conductivity, moisture content, free acidity, HMF and minerals (12). In market, there are various types of unbranded and branded honeys available. In terms of nutritional value and quality, significant differences may exists in various brands of the honey. Majority of the population have no information about the quality of honey they use. Due to increasing problems related to honey tampering and adulteration, it is necessary to cheer quality testing of branded and local honey available in the market. Thus, the present study was aimed to determine the honey quality of different brands of honey present in the market of Lahore, Punjab (Pakistan) and compare it with Pakistan Standards and Quality Control Authority (PSQCA) and International honey standards for the Codex Alimentarius to obtain a valuable data for the honey users (13, 14).

MATERIAL AND METHODS

This study was carried out in research and development laboratory of Islamic Shehad centre (Pvt) Ltd. Lahore. This study was planned to test the honey quality of 17 brands (both national and international) obtained from local market of Lahore. Samples of honey were investigated for electrical conductivity, HMF, total reducing sugars, sucrose, pH, acidity, moisture, Fiehe's Test, Water and mineral content (Ash) and compared with PSQCA and International honey standards for the Codex Alimentarius (13, 14).

Determination of pH and acidity

In 250 ml beaker, 75 ml of distilled water (CO_2 free water) was taken and 10 gram of honey sample was dissolved in it. Then the pH was measured by using pH meter (901, Bante USA), standardized at pH 4.0, 7.0 and 10. For acidity, 0.1 M sodium hydroxide (NaOH) was used to titrate this solution till pH became 8.3 (14).

Acidity (m.eq/kg) = weight of Honey × Vol of NaOH used

Determination of Electrical conductivity (E.c)

Conductivity meter (901, Bante USA) was used to determine E.c. Standard solution was used to calibrate the electrical conductivity meter. Then in 10% solution of honey conductance cell of electrical conductivity meter was immersed. After stabilization of instrument, reading was noted.

Determination of Moisture content

Refractometer (IR 120, INSMARK CHINA) calibrated with distilled water was used to check the moisture content of honey samples. A sample from each brand of honey was placed on clean and dry prism of refractometer. Reading was noted and compared with standard table of Association of analytical chemists (AOAC) to obtain moisture percentage (15).

Determination of Mineral (Ash) Content

Clean Ash dish was taken and dried in electrical furnace (SX-2.5-12, Vincom China) at 550°C. Then dish was weighed (M2) after cooling it at room temperature using desiccator. In this ash dish
5 grams of honey (M0) was added. To prevent frothing ash free olive oil (2 drops) was added in this. To char the sample, bunsen burner was used. Then after placing sample dish in preheated muffle furnace, it was heated at 550oC for 1 hour. The ash dish was weighed after cooling. This process (ashing) was continued till white color ash was obtained (M1). Using the formula of Codex Alimentarius, the percentage (%age) of ash in g/100 honey was obtained (14).

$$Ash=(M1-M2)/M0\times100$$

Where: M0 = honey weight taken in grams

M1= dish weight in grams + weight of ash in grams weight and

M2 = dish weight in grams

Total reducing sugar content (before and after inversion)

Soxhlet's modification of Fehling's solution was used to determine reducing sugars in honey. Lane, (1923) developed this method and in Codex Alimentarius this method was also included (14,16).

Reducing sugar before inversion

Using a volumetric flask of 250 ml, distilled water was used to dissolve 2 gram of honey (W2). Dilution was done by adding 200 ml water to make a honey solution. Then from this honey solution 50 ml was taken and further diluted by

adding 100 ml distil water in it (diluted honey solution). From this diluted honey solution, 50 ml was taken in a burette. Then in a 250 ml flask, 5ml each of Fehling's solution A and B was added. After the addition of the 7-8 ml distilled water, flask was heated on a burner till it starts boiling. Titration was completed during boiling. 0.2 % ofmethylene blue (1 ml) was used as an indicator. The end point of the reaction was considered when there was a color transformation of the solution (i.e. blue to colorless). Using following formula, % age of total reducing sugar before inversion (R.B.I) was noted

C=2/W2×1000/Y2

Where: C = total reducing sugar/gram (before inversion)

W2= weight of sample (honey) taken in gramsY2=

volume of diluted honey used in ml **Reducing**

Sugar after inversion

50 ml honey solution (taken from preparedsolution for determination of total reducing sugar before inversion) together with 25 ml distilled water was added in a graduated flask. Thissolution was heated on a water bath at 65°C and then removed. 10 ml of hydrochloric acid (HCl) was added after removal of flask from the waterbath and permitted to cool at room temperature for fifteen minutes. And then set the temperature at 20°C. Using litmus paper as indicator, neutralization of this solution was done by NaOH. Again solution was allowed to cool, and then diluted by adding (100ml) distilled water (diluted honey solution). Then in a 250 ml flask, 5 ml each of Fehling's solution A and B was added. Afterthe addition of the 7-8 ml distilled water, flask was heated on a burner till it starts boiling. Titrationwas completed during boiling. 0.2 % of methylene blue (1 ml) was used as an indicator. The end point of the reaction was considered when there was a color transformation of the solution (i.e. blue to colorless). Using following formula, % age of total reducing sugar after inversion (R.A.I) was noted

C=2/W2×1000/Y2

Where: C = total reducing sugar/gram (after inversion)

W2= weight of sample (honey) taken in gramsY2 =

volume of diluted honey used in ml Sucrose content

was noted as follow

The % age of sucrose was calculated as follow:

Sucrose content (%age) = (R.A.I– R.B.I) × 0.95

Hydroxy methyl furfural (HMF)

5 gram of honey was dissolved in a small beaker (weighed to the nearest 1 mg in a small beaker). This solution was diluted by adding 25 ml of distilled water by transforming it into 50ml volumetric flask. Then 0.5 ml of each Carrez solution I and II was added to this solution and diluted again with distill water (to make a total volume of 50 ml). To suppress foam formation a drop of HCl was added. Then this solution wasfiltered using filter paper. First 10 ml filtrate was discarded and from remaining filtrate 5 ml filtrate was pipetted into each of 2 test tubes. Distilled water (5 ml) was pippeted into one (sample) and 0.20% sodium bisulphate (5ml) into the other (reference). Mixed well and by using the UV spectrophotometer (Halo DB-20 Dynamica UK) the absorbance of the sample against thereference was measured at a wavelength of 284nm and 336. Result were expressed in mg/kg using following equation (15).

HMF in mg/kg = ((A284-A336)×74.87×10)/W

Where: W= weight of sample (g) takenA284=

Absorbance at 284 nm

A336 = Absorbance at 336 nm

Constant Factor=(126×100×1000×100)/ (16830×1000) = 74.87

10= conversion of gram into mg

ADULTERATION CONFIRMATION TEST

Fiehe's Test

It is a qualitative test based on the detection of HMF. HMF is formed by the dehydration of fructose which is obtained by the acidic hydrolysis of sucrose. The furfural reacts with the resorcinol, forming a color. If the color formed is red, then itis confirmed as positive (17). If there is no color or

a pinkish color that disappear with time, then the test confirms negative.

RESULTS

Results of physiochemical characteristics of 18 honey samples from local market of Lahore, Punjab (Pakistan) are reported in Table 1.The results show that the pH of honey samples investigated was acidic in nature, ranging from 3.09 to 5.84 with a mean of 4.70 (Table 1). The free acidity as recorded in this study ranged from 14.02 to 33.4 meq/kg with a mean of 23.21 meq/ kg (Table 1). The electric conductivity values of the honey samples varied (as shown in Table 1)in the range 0.1 - 0.6 mS/cm with a mean value of 0.27 mS/cm (Table 1). The moisture content of the honey sample as shown in Table 1 revealed a range between 14.5 to 25.7 % with a mean value of 18.29%. One sample has more value (25.7%) as compared to standards value. The total sugar of honey samples studied was ranged from 66.2 to 80.1 % with mean value of 74.78 % (Table 1). Sucrose content of honey samples ranges from 1.33 to 14.2 % with a mean value of 5.84 %. The mineral (Ash) values obtained were between 0.002 to 0.234 % with a mean value of 0.104% (Table 1). HMF of the selected honey samples ranged from 14.5 to 112 mg/kg with a mean value of 59.95 mg/ kg (Table 1). The adulteration detection was done by Fiehe's test. Fiehe's test was positive in case of 7 samples (38.88%) as shown in Table 1.

DISCUSSION

The results of different parameters of all the honey samples collected from the markets of Lahore were compared with PSQCA and Codex Alimentarius Standards (13, 14). It was observed that pH, acidity, electrical conductivity, reducing sugar and mineral content (ash) of all samples are within normal ranges, whereas moisture content, sucrose, and HMF were not within standard range.

The moisture content of the honey sample as shown in Table 1 revealed a range between 14.5 to 25.7 %. All honey samples except honey sample IAJ have moisture content within the standards limit of PSQA and Codex Alimentarius (Not more than 21%). Moisture content contributes to honey quality, its viscosity, fermentation and savor and is one of the basic quality characteristic of honey (18). Honey has an excellent tendency to absorb atmospheric moisture (hygroscopic property) and thus readily increase its moisture levels. Extraction and harvesting methods of honey (varied due to location, practices and species) may also increase the moisture content (12).

Fructose and glucose are the two main reducing sugar which are usually present in honey, giving it characteristics similar to invert syrup (19). The results of this study indicated that reducing sugar of tested samples were between 66.2 to 80.1 %with mean value of 74.78 %. All of the samples met the quality criteria of national and international standard.

It is known that sucrose level of honey varies according to the origin of nectar compounds and degree of maturity of honey. Level of sucrose in a honey is used as a parameter to detect adulteration (addition of cane or other sugars) of honey. By measuring sucrose content one can detect added sugar in honey (1). 5 % sucrose is the maximum prescribed limit as per the PSQCA and Codex Ailmentarius (13, 14). The sucrose (%) content in 18 honey sample analyzed ranged from 1.33 to 14.2 %. Out of 18 samples 7 samples (38.88%) had high sucrose content, which indicates that the honeybees are over fed with sugar syrup, early harvesting or any adulteration (20, 21). HMF is formed when fructose is decomposed. It mainly depends upon storage period, heating temperature and pH. The higher the HMF value, the lower the quality (3). The Codex Alimentarius and PSQCA recommended a maximum of 80 mg/kg for HMF. In this study, the HMF is ranged between 14.5 to 112 mg/kg, which is not within the standard range. In Table 1 seven samples showed more than 80 mg/kg of HMF. It means these samples are not good quality honey because of more shelf life or heating on high temperature for a longer period of time. Fiehe's test was performed to detect if there are any adulterants in the honey samples obtained from local market of Lahore (17). Fiehe's test was positive (samples were adulterated) in case of 7 samples (38.88%) as shown in Table 1.

CONCLUSION

Generally, the findings showed that attributes of 10 (55.5%) out of 18 of the commercial honeys meet with national and international standards, which make them good for human consumption. However while others have deviated from national and international standards with the parameters like sucrose content, HMF and moisture parameters thus suggesting adulteration and render the honey unhealthy for children andadults.

Table 1 ; Comparison of physiochemical properties of Branded Honey collected from Local market of Lahore and compared to National and International Standards

Brands	pH	Acidity (meq/kg)	E. C (mS/cm)	Moisture (%)	Reducing sugar (%)	Sucrose (%)	Mineral / Ash (%)	Fiehe's Test	HMF (mg/kg)
IAA	3.73	26.5	0.2	18.4	76.1	3.8	0.221	Negative	35.3
IAB	4.50	28.1	0.11	17.2	77.7	2.5	0.003	**Positive**	**83.1**
IAC	4.91	33.4	0.2	19.4	69.0	4.6	0.091	Negative	14.5
IAD	5.51	22.0	0.2	18.4	67.0	2.5	0.234	Negative	38.4
IAE	3.84	19.5	0.32	16.2	72.5	3.8	0.063	Negative	**108.6**
IAF	3.82	28.5	0.2	19.4	77.2	**11.1**	0.035	**Positive**	**81.12**
IAG	5.80	15.0	0.1	20.1	74.2	**5.6**	0.150	**Positive**	**92.54**
IAH	5.08	16.4	0.15	17.8	80.1	**9.1**	0.002	**Positive**	61.07
IAI	4.43	23.7	0.3	19.4	79.6	3.1	0.107	Negative	28.03
IAJ	5.06	14.02	0.6	**25.7**	66.2	**14.2**	0.151	**Positive**	18.06
IAK	4.13	27.2	0.4	16.4	78.5	1.8	0.083	Negative	**88.6**
IAL	5.84	25.2	0.5	17.5	77.2	**12.5**	0.137	**Positive**	34.2
IAM	5.03	16.3	0.2	18.2	71.7	4.5	0.127	Negative	48.6
IAN	4.92	23.5	0.31	16.0	73.03	3.2	0.004	Negative	**112.5**
IAO	4.74	24.8	0.42	14.5	69.6	**5.7**	0.080	Negative	75.89
IAP	5.52	28.5	0.3	18.2	75.4	**13.1**	0.175	**Positive**	**82.0**
IAQ	4.70	27.6	0.2	19.1	77.2	2.7	0.126	Negative	22.6
IAR	3.09	17.6	0.19	17.4	74.89	1.33	0.090	Negative	56.0
Mean Value	4.70	23.21	0.27	18.29	74.28	5.84	0.104	-	60.05
Range	3.09-5.84	14.02 -33.4	0.1-0.6	14.5-25.7	66.2-80.1	1.33-14.2	0.002-0.234	-	14.5-112
National (PSQ-CA)	3.9-6.1	NMT 40	----	NMT 21%	NLT 65%	NMT 5 %	NMT 0.6%	Negative	NMT 80
Interna-tional	3.42-6.10	NMT 40	0.2 - 1.8	18-23	NLT 60	NMT 5 %	0.25-1.0	------	NMT 80

E.c=Electrical conductivity, NMT= Not more than, NLT= Not less than

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