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A research on the chemical and microbiological qualities of honeys sold in Istanbul

Harun URAN¹, Filiz AKSU^{2*}, Dilek DÜLGER ALTINER³

Abstract

Honey, produced by honeybees from nectar in flowers and plants, is an aqueous supersaturated sugar solution, mainly composed of fructose and glucose. The quality and biochemical properties of honey are related to honey maturity, production methods, processing and storage conditions, climatic conditions as well as the nectar source of the honey. In this study, 35 honey samples of different sizes and packaging all of which were sold in Istanbul were examined in terms of some physicochemical and microbiological properties. All samples were floral liquid honey. The physicochemical characterizations were as follows: average moisture ($16.31 \pm 1.40\%$), ash ($0.30 \pm 0.29\%$), hydroxymethylfurfural (HMF) (8.09 ± 7.63 mg/kg), pH (4.32 ± 0.34), acidity (30 ± 9 meq/kg). In addition, total mesophilic aerobic bacteria, *Enterobacteriaceae*, yeast and mold analyses were performed in these samples. According to the microbiological analyses; the average total number of mesophilic aerobic bacteria was 2.68 log cfu/g. *Enterobacteriaceae* have not been observed in the samples. However, in three samples, mold-yeasts were detected at the levels between 1.30 and 1.60 log cfu/g.

Keywords: chemical quality; HMF; honey; microbiological quality.

Practical Application: The quality of honeys are related to their physicochemical and microbiological properties.

1 Introduction

Honey is a natural product made by honeybees from the nectar of plants, secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect, transform by combining with their peculiar substances, dehydrate, leave and store in honeycombs to ripen and mature (Turkey, 2012). It is produced in three different forms such as blossom or nectar honey, honeydew and comb honey (Turkish Standards Institution, 2010). Honey is chemically composed of sugar (70-80%), water (10-20%), organic acids, minerals, vitamins, protein, phenolic compounds and free amino acids. The major monosaccharide ingredients of honey are glucose and fructose. It is acidic and pH value ranges between 3.5 and 5.5 (Aydin et al., 2008). Honey may also be considered as a nutrient since it contains α -tocopherol, ascorbic acid, flavonoids and phenolic compounds (Mendes et al., 1998).

Formation of honey depends upon physiological, chemical and enzymatic activities. The composition of honey is generally associated with two factors. The most important factor is the source of nectar and the latter is the extrinsic factors. Extrinsic factors consist of local climatic factors, type of soil, altitude and production methods of the beekeepers (Uçkun & Selli, 2012). The quality of honey is mainly associated with its botanical source and chemical properties. Honeys that are of different botanical and regional origin contain different constituents (Cinar, 2010).

Anklam (1998) reported that there was no forthright parameter available to assess the biological activity and the

actual quality of honey and routine chemical tests were far from providing sufficient data with respect to the quality of honey. Diastase activity and HMF (Hydroxymethylfurfural) constituent of honey are the indicators widely used to assess the freshness of honey even though there is an ongoing debate about the effects of this substance on human health (Kuçuk et al., 2007). HMF content in honey depends on processing method and storage conditions. According to the guidelines of legislation, maximum HMF concentration allowed in honey is 40 mg/kg in our country (Turkey, 2012).

There are miscellaneous research studies regarding honeys sold in different regions of Turkey. In these studies honey samples were investigated in terms of their accordance with the standards and the regulations (Akyuz et al., 1995; Yilmaz & Kufrevioğlu, 2001; Unal & Kuplulu, 2006; Aydin et al., 2008). Moreover there are several studies with respect to the quality of honeys sold in different regions of the world (Finola et al., 2007; Isla et al., 2011; Ajlouni & Sujirapinyokul, 2010; Mendes et al., 1998; Feas et al., 2010; Anupama et al., 2003; Soria et al., 2004; Al et al., 2009).

In our study, a total of 35 honey samples sold by different manufacturers were investigated in terms of various physicochemical and microbiologic properties. Data obtained were compared with the notifications declared by Turkish Food Codex concerning Honey Regulation (Turkey, 2012) and Honey Standards of Turkish Standards Institution (2010).

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2 Materials and methods

2.1 Sampling

Samples of strained honey were purchased in their original packaging from different districts of Istanbul and stored at 4°C in the laboratory until the analyses. Homogenous samples were harvested from each specimen and the analyses were carried out in a parallel manner.

2.2 Physicochemical analyses

Chemical parameters such as moisture, ash, pH, acidity, and HMF were analyzed to assess the chemical composition of the honey samples. Moisture and ash contents were determined on the basis of total weight loss principle of foodstuff (Association of Official Analytical Chemist, 1990). So as to determine pH level, 10 g of honey was dissolved in 75 mL distilled water and measured by Hanna pH meter. (Association of Official Analytical Chemist, 1990). Total acidity was assessed by titration method and expressed as malic acid (Association of Official Analytical Chemist, 1990). Spectrophotometric analyses was performed to determine HMF content. Two mL of diluted honey samples were collected in test tubes and 5 mL of p-toluidin was poured onto each tube. One tube was kept as the reference solution and added 1 mL of pure water and the second tube, which was the sample solution, was added 1 mL of barbituric acid. All tubes were properly stirred and absorbance of sample solution versus reference solution was read at 550 nm wavelength. HMF contents of the samples were assessed on the previously drawn standard curve diagram (Cemeroglu, 2007).

2.3 Microbiological analyses

Complying with the rules to be obeyed during sterile sampling, 10 g of each specimen was homogenized in 90 mL (1:10 dilution) of 0.1% saline peptone water (Oxoid CM 733R) for 2 min (Seward Lab Blender 400, Worthing, England) and a series of dilutions were prepared up to 10^{-8} level. (Thaddeus et al., 2001). Then microbiological inoculation of the dilutions was performed on common and selective growth media to estimate total count of mesophilic aerobic bacteria, *Enterobacteriaceae* and mold-yeast.

Standard plate count agar (Oxoid CM 463) was used to obtain total count of mesophilic aerobic bacteria and for this purpose, the petri plates were incubated at 37°C for 48 h prior to evaluation (Maturin & Peeler, 2001).

Violet Red Bile Glucose Agar (Oxoid CM 485) was applied to estimate the count of *Enterobacteriaceae*. After the inoculation, the plates were covered with an extra layer of medium, stirred and then incubated at 37 °C for 24 h. Red coloring with a diameter of 0.5 mm and larger were evaluated as *Enterobacteriaceae* colonies (International Organization for Standardization, 2004).

So as to estimate mold-yeast count, Potato Dextrose Agar (PDA) (Merck) medium was sterilized and acidity was achieved by adding 10% lactic acid solution (pH 3.5±0.1) and then the inoculated petri dishes were incubated at 25 °C for 5 days (Tournas et al., 1998).

3 Results and discussion

Physicochemical and microbiological findings concerning honey samples were summarized in Table 1 and Table 2.

On the basis of our findings, mean values for moisture and ash contents, total acidity and pH levels of the tested honey samples were 16.31%, 0.30%, 30 meq/kg and 4.32, respectively. According to the current Honey Standard and the Regulation, maximum levels of moisture, ash, acidity and pH for blossom/flower-based honey were declared to be 20%; 0.6%; 50 meq/kg and 6.1 (Aydin et al., 2008; Turkey, 2012). Our results for these physicochemical parameters were in accordance with the Standards.

Based on the results, HMF content ranged between 1.07 mg/kg and 30.69 mg/kg. 40 mg/kg is the maximum level permissible for HMF in the relevant Standards and the Regulation (Aydin et al., 2008; Turkey, 2012). Concerning this permitted maximum level, HMF content was also in accordance with the standards.

Microbiological analyses revealed that average count of total mesophilic aerobic bacteria (TMAB) was 2.68 log cfu/g and mean value for mold-yeast amount was below 1.00 log cfu/g level. There was no evidence of *Enterobacteriaceae* in none of the samples.

The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH, the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey and phytochemical factors (Mahendran & Kumarasamy, 2015). So It is highly unlikely to grow vegetative forms of bacteria in honey due to its antibacterial properties (Aydin et al., 2008). On the other hand, mold-yeast growth was demonstrated at 10^2 to 10^3 cfu/g levels in some honey samples (Rall et al., 2003; Iurlina & Fritz, 2005; Aydin et al., 2008). Mold-yeast count markedly affects the shelf life of honey products. Fermentation caused by osmophilic yeasts that grow at low pH levels is responsible for the spoilage of honeys (Aydin et al., 2008).

Table 1. Physicochemical analyses findings of the honey samples.

Analyses	Minimum	Maximum	Mean	Standard Deviation
Moisture (%)	14.11	19.66	16.31	1.40
Ash (%)	0.009	0.80	0.30	0.29
Total acidity (meq/kg)	13.50	46.50	30.0	9.0
pH	3.75	5.19	4.32	0.34
HMF (mg/kg)	1.07	30.69	8.09	7.63

Table 2. Microbiological analyses findings of the honey samples (log cfu/g).

Analyses	Minimum	Maximum	Mean	Standard Deviation
TMAB	1.30	3.38	2.68	0.54
<i>Enterobacteriaceae</i>	<1.00	<1.00	<1.00	-
Mold-Yeast	<1.00	1.54	<1.00	0.17

There are numerous studies carried out with honey samples obtained from the different regions of Turkey. Akyuz et al. (1995) investigated the accordance of physical and chemical properties of 20 honey samples which were sold in different districts of Van, with the Standards and the mean values of the investigated parameters were as 17.80%, 0.18%, 24.61 meq/kg, 4.11, 25.87 mg/kg for moisture, ash, total acidity, pH and HMF, respectively. These findings were in accordance with the Standards and compatible with ours.

In a study, 70 strained honey samples (35 blossom and 35 honeydew honeys) served for consumption in Ankara were investigated in terms of certain chemical properties. Moisture content, ash content, HMF ranged between 13% and 25%; 0.11% and 0.71%; 11.13 and 256.27 mg/kg, respectively which actually did not comply with the Standards and the Regulation. Total acidity varied from 8.23 to 33.21 meq/kg, which was in accordance with the standards. In conclusion 80% of honeydew and 31.43% of blossom honey samples did not comply with Turkish Food Codex Honey Regulation (Unal & Kuplulu, 2006).

Aydin et al. (2008) performed a similar study and investigated the chemical and microbiological properties of 20 honey samples sold in Kars region. According to their findings, pH value, moisture content and total acidity ranged between 2.21 and 3.54; 13.2% and 19.2%, and 6 and 22.5 meq/kg, respectively. These findings complied with the standards and the Regulation. However, HMF levels varied from 2.49 to 205.15 mg/kg and some samples deviated from permissible ranges. According to microbiological analyses, three of the samples contained lactic acid in a range between 10^2 and 10^3 cfu/g. Another three of the samples contained *staphylococci* at the levels of 10^2 - 10^4 cfu/g. Mold-yeast were detected in 10^2 - 10^3 cfu/g levels in eight of all samples.

Kahraman et al. (2010) performed a similar study and investigated physico-chemical parameters of 70 honey samples obtained from retailers in Marmara Region and East Anatolia of Turkey. According to their findings, 10 of 70 (14.3%) honey samples were of unacceptable quality based on recommended criteria of moisture (3 of 70, 4.29%), diastase activity (3 of 70, 4.29%), HMF (2 of 70, 2.86%), invert sugar (4 of 70, 5.71%) and sucrose (2 of 70, 2.86%), by Turkish Food Codex and European Commission Regulation. The results of this study indicated that 85.7% of honey samples were at good quality.

In another study, 45 honey samples manufactured in eastern and southeastern parts of Turkey were evaluated in terms of the effects of their chemical compositions and storage conditions on HMF content and diastase activity. The results complied with the standards. After 1 year of storage, HMF content increased while diastase activity was reduced (Yilmaz & Kufrevioğlu, 2001).

Similar studies are available in different countries worldwide. Finola et al. (2007) from Argentina collected 23 fresh honey samples sold in the region with the highest population and performed chemical and microbiological analyses. Chemical parameters such as HMF, glucose+fructose level (sugar profile), water content, ash content and total acidity were found to be extremely low, 72.8%, below 20%, 0.063% (mean value) and 20.6 meq/kg (mean value), respectively. Mold-yeast level was below an average 10 cfu/g. We may conclude that the quality

of the honeys were markedly high, which was consistent with that of ours.

In Australia, Ajlouni & Sujirapinyokul (2010) evaluated the honeys of different manufacturers in terms of HMF and amylase contents. Chemical properties of the honeys significantly varied among the samples. pH, total acidity and moisture content ranged between 4.02 and 4.69; 33.5 and 53.5 meq/kg; and 10.6% and 17.8%, respectively. The findings showed that heating process was not the only factor that increased HMF content in honey. The composition of the honey, pH value and the source of honey all contributed to this change. In conclusion it was noted that HMF content was not the single parameter concerning the quality of the honey.

Another country in which significant studies regarding honey were performed is Portuguese. Mendes et al. (1998) investigated the quality parameters of 25 honey samples and assessed carbohydrate levels by HPLC method. Chemical parameters evaluated were HMF level, moisture and ash contents, diastase activity, free acidity and the amount of insoluble materials. They also carried out organoleptic examinations. In conclusion only 13 honeys complied with the national and international notifications and the remaining 12 were not in accordance with the regulations in terms of at least one or more parameters.

Some studies were performed also in India. Anupama et al. (2003) investigated the physicochemical and organoleptic properties of 11 honey samples of different botanical and regional source, which were produced and sold in different seasons and under various circumstances. In conclusion, they detected that moisture content, pH value, viscosity, acidity and total reducing sugar and finally sucrose content of the samples ranged between 17% and 22.6%; 3.62 and 5.46; 1.79 and 13.8 Pas; 0.03% and 0.15%; 61.3% and 72.6% and 1.2% ile 5.7%, respectively.

4 Conclusion

In our study, physicochemical properties of analyzed commercial honeys were all in accordance with Turkish Standards Institution Honey Standards and Turkish Food Codex Honey Regulation. Microbiological load, as well, was within the permissible range. Presence of certain amount of bacteria in the samples is considered to be associated with the contamination during straining, transportation, packaging, storage or marketing.

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