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Microbiological and Physicochemical Characterization of Honey Samples from Erzurum

Erzurum İline Ait Bal Örneklerinin Mikrobiyolojik ve Fizikokimyasal Karakterizasyonu

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ABSTRACT

In this study, the physico-chemical and microbiological characteristics of 20 honey samples stored at room temperature and collected from Erzurum province (Turkey) was studied. Moisture content varied from 13.80% to 20.60%, refractive index from 1.4850 to 1.5023, ash value from 0.026% to 0.495%, pH from 3.11 to 4.58, total acidity from 14.61 meq/kg to 53.44 meq/kg, total sugars from 63.89% to 86.49%, reducing sugars 53.38% to 78.29% and sucrose from 0.45% to 21.66%, diastase activity from 0 to 38.5 °G, 5-Hydroxymethylfurfural from 0.77 to 5.76 mg/kg. The color parameters L^*, a^* and b^* determined using a colorimeter were within the range of 36.04–57.12, –1.92 to 7.46, and 2.69–22.91, respectively. The least and the highest of total aerobic mesophilic bacteria, fungi (yeasts and molds), and osmophilic yeast counts were determined to be 1.5×10 to 1.3×10^3, <10 to 2.6×10^2, <10 to 4.0×10^2 cfu/g, respectively. However, thermophilic *Bacillus* spores and coliform bacteria counts were found to be <10 cfu/g in analyzed samples.

Keywords: Color, diastase activity, HMF, honey, microbiological properties, sugar

ÖZ

Bu çalışmada, oda sıcaklığında saklanan ve Erzurum ilinden toplanan 20 adet bal örneğinin fizikokimyasal ve mikrobiyolojik özellikleri incelenmiştir. Örneklerin nem içeriği %13,80 ile %20,60 arasında, kırılma indisi 1,4850 ile 1,5023 arasında, kül değeri %0,026 ile %0,495 arasında, pH 3,11 ile 4,58 arasında, toplam asitlik 14,61 meq/kg ile 53,44 meq/kg arasında, toplam şekerler %63,89 ile %86,49, indirgen şekerler %53,38 ile %78,29 ve sükroz %0,45 ile %21,66, diastaz aktivitesi 0 ile 38,5 °G, 5-Hidroksimetilfurfural 0,77 ile 5,76 mg/kg arasında değiştiği tespit edilmiştir. L^*, a^* ve b^* renk parametreleri sırasıyla 36,04-57,12-1,92 ile 7,46 ve 2,69-22,91 aralığında belirlenmiştir. Toplam aerobik mezofilik bakteri, maya ve küf ve ozmofilik maya sayılarının en az ve en yüksek değerleri sırasıyla $1,5 \times 10$ ile $1,3 \times 10^3$, <10 ile $2,6 \times 10^2$, <10 ile $4,0 \times 10^2$ cfu/g olarak belirlenmiştir. Diğer taraftan analiz edilen örneklerde termofilik Bacillus sporları ve koliform bakteri sayılarının <10 kob/g olarak belirlenmiştir.

Anahtar Sözcükler: Renk, diastaz aktivitesi, HMF, bal, mikrobiyolojik özellikler, şeker

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Introduction

Honey, a viscous, aromatic native sweet product, is manufactured by *Apis mellifera* bees from the nectar of herbs or from 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 specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Golob et al., 2005). The essential types of honey according to botanical source are flower honey or sap honey, obtained from the nectar of plants, and honeydew honey (Golob et al., 2005; Muñoz and Palmero, 2006). Honey is a widely used food that liked around the world since the earliest times. Honey contains at least 181 substances (Ouchemoukh et al., 2005). It is a semi-solid food (water: 15–18% approx.) which comprises a complex mixture of carbohydrates, mainly glucose and fructose; other sugars are present as traces, depending on the floral origin. Moreover, organic acids, lactones, free amino acids, minerals, vitamins

(vit B_1, B_2, C, and nicotinic acid), phenolic compounds, enzymes, and solid particles, mainly consisting of pollen, traces of wax, and variable amounts of sugar-tolerant yeast and pigments are present (Downey et al., 2005; Fallico et al., 2004; Ouchemoukh et al., 2005; Özcan et al., 2006). Bees and plants are the sources of honey components, and honey composition depends to a great extent on the nectar sources, plant, climate, environmental conditions, and the ability of the beekeeper (Fernández-Torres et al., 2005; Özcan et al., 2006).

Besides its nutritional value, honey also contains bacterial agents with different floral activity and a limited variety of microorganisms. Spore-forming aerobes and anaerobes are the dominant microflora in honey. In addition, lactic acid bacteria (LAB), yeast, and fungi species can be found in honey either as a part of the existing microflora or as a result of contamination (Lani et al., 2017). Vegetative forms of bacteria that cause human disease have not been found in honey. Since bacteria do not multiply in honey, the high number of vegetative bacteria in honey indicates recent contamination from a secondary source (Iurlina & Fritz, 2005). Microbial contamination occurs in honey from various sources. Primary sources of contamination are pollen, water, digestive system of a honey bee, powder, air, soil, and nectar. These are relatively difficult to eradicate. Secondary microbial infections occur during postharvest processing and storage of honey. It is due to the lack of attention to hygiene, which can be controlled more easily with good production practices (Lani et al., 2017). Major microbial contaminants in honey are molds, yeasts, *Bacillus* spp., and *Clostridium* spp.; their numbers are indicators of the commercial quality and safety of honey. Honey has natural antimicrobial properties that can delay develops many microbes (Lani et al., 2017). Microorganisms in honey may influence quality or safety. These also could cause human illness under certain conditions (Migdał et al., 2000; Snowdon & Cliver, 1996). Osmophilic yeasts are a problem in the honey industry for starting the fermentation of honey. As fermentation is proportional to the concentration of yeast, honey with a very high yeast count is not likely to be palatable or marketable. Honey made from flowers in humid regions has more yeast and can spoil in the comb (Snowdon & Cliver 1996).

No published information on the microbiological properties of honey collected from Erzurum is available. For this reason, the aim of this work was to determine the chemical and microbiological properties of honey stored at room temperatures and collected from Erzurum. The following parameters were analyzed: refractive index, moisture, ash, sucrose, total and reducing sugars, total acidity, pH, diastase activity, 5-hydroxymethylfurfural (HMF), color parameters (L^*, a^* and b^*), which are considered the basic parameters for characterizing honey. On the other hand, total aerobic mesophilic bacteria, fungi (yeasts and molds), osmophilic yeast counts, thermophilic *Bacillus* spores, and coliform bacteria were also determined. Thus, results obtained from this work would probably help to introduce the honey.

Methods

Honey Samples

The present study used 20 honey samples collected in Erzurum (Turkey). The samples were taken directly from the beekeepers. All samples were unpasteurized, stored in glass sample jars, and immediately transferred to the laboratory and kept at room temperature until required for analysis. Analyses were carried out at least in duplicate.

Chemical Analysis

Moisture was determined by measuring the refractive indices at 20°C with a Carl Zeiss Abbé refractometer, and the corresponding moisture content (%) was calculated using the Wedmore table (AOAC, 1984). The following parameters were determined according to AOAC methods (AOAC, 1984): ash, diastase activity, total acidity, and pH. Total acidity and pH were measured using an ATI-ORION 420A pH meter. 5-Hydroxymethylfurfural was measured by a spectrophotometric method (Cemeroğlu, 2010). Sucrose and total and reducing sugars were estimated by Lane and Eynon method.[9] L^*, a^*, and b^* color parameters were determined using the Minolta colorimeter (CR-200 Minolta Camera Co., Osaka, Japan). L^* is the luminance or lightness component, which ranges from 0 to 100, and a^* and b^* are the two chromatic components which a: +: red; −: green and b: +: yellow; −: blue.

Microbiological Analysis

Homogenization of Honey Samples

Eleven grams of honey were homogenized for 1.5 minutes with a Stomacher Lab-Blender in 99 mL of 2.0 % sodium citrate solution. Serial dilutions were prepared in 0.1 sterile peptone water, and duplicate plates were used for all microbiological counts.

Microorganisms and Growth Conditions

In the microbiological analysis studied in honey, the media and conditions of incubation used were as follows: (a) Total aerobic mesophilic bacteria, plate count agar (PCA, Oxoid), 30 ± 1°C/48 ± 3 h (Baumgart et al., 1993). (b) Fungi (yeasts and molds), Yeast Exract Glucose Chloramphenicol Agar FIL-IDF (YGC Agar, Fluka), 25 ± 2°C/3–5 days (Baumgart et al., 1993). (c) Thermophilic Bacillus spores, Casein-peptone Soyameal-peptone Agar (CASO Agar, Fluka) (dilutions incubated 70 ± 1°C/10 minutes), 55 ± 1°C/72 ± 3 h (Baumgart et al., 1993). (d) Coliform bacteria, Violet Red Bile Agar (VRB Agar, Merc), 24–48 ± 1°C/24 ± 3 h (Baumgart et al., 1993). (e) Osmophilic yeasts, Potato Dexrose Agar (PDA, Merc) (added 60 % saccharose), 32 ± 1°C/48 ± 3 h (Baumgart et al., 1993).

Statistical Analysis

To estimate variability in chemical composition and microbiological properties of honey samples were determined some statistical parameters including mean, range, standard deviation, and coefficient of variation for each index Additionally, correlation was used to determine the possible relationship between the (a) growth of microbial groups, and (b) presence of indicator and pathogen (SPSS, 1999).

Results, Discussion and Conclusions

Physicochemical Parameters

Table 1 summarizes the minimum, maximum, mean, standard deviation, and variation coefficient of the data obtained from analyses of the selected physicochemical parameters (refractive index, moisture, ash, pH, total acidity, diastase activity, HMF, reducing sugar, sucrose, total sugar, L^*, a^* and b^* color parameters).

The moisture content of honey depends on the harvest season, climate, moisture content of original plant nectar, degree of maturity reached in the hive, processing techniques, and storage conditions, and it is highly important for the shelf life of the honey during storage (de Rodríguez et al., 2004; Downey et al., 2005; Ouchemoukh et al., 2005; Özcan et al., 2006). A high moisture content generally causes the honey to ferment, spoil, and lose flavor, causing honey-quality loss. The refractive index varied from 1.48 to 1.50, and the corresponding moisture content ranged

Table 1.
Some Physical and Chemical Characteristics of Honey Samples

Physicochemical Parameters	Minimum	Maximum	Mean	SD	Variation Coefficient
Refractive index	1.49	1.509	1.50	4.78	319.76
Moisture (%)	13.80	20.60	16.28	1.95	12.02
Ash (%)	0.03	0.50	0.12	0.11	91.08
HMF (mg/kg)	0.77	5.76	3.19	1.52	48.01
Reducing sugar (%)	53.38	78.29	65.80	5.65	8.60
Sucrose (%)	0.45	21.66	7.87	6.33	80.53
Total sugar (%)	63.89	86.49	73.67	5.50	7.47
pH	3.11	4.58	3.69	0.42	11.48
Total acidity (meq/kg)	14.61	53.41	27.42	10.92	39.83
Diastase activity (°G)	0	39.00	20.45	11.88	58.09
L^*	36.04	57.12	47.023	6.73	58.09
a^*	–1.92	7.46	0.98	2.49	256.25
b^*	2.69	22.91	13.36	5.55	41.58

Note: G = Gothe; SD = Standard deviation.

between 13.80% and 20.60%; these values are within the range found by other researchers (Lazaridou et al., 2004; Ouchemoukh et al., 2005; Sanz et al., 2005; Yanniotis et al., 2006). Eighteen samples yielded moisture between 13.80 % and 20.00 %, indicating a proper degree of maturity. Nevertheless, two samples had moisture contents slightly above 20%, likely due to earlier honey extraction from hives. More samples with moisture contents were below 20%; the maximum value allowed by Turkish Food Codex, Honey Notification (2000) means that the fermentation ability is low. Usually, the quality of the studied honey is good because honey samples have low moisture content.

Sugars represent the largest portion of the honey composition, and the monosaccharides fructose and glucose are the most abundant, while small amounts of disaccharides (mainly maltose and sucrose) are also present; other disaccharides higher sugars (trisaccharides and oligosaccharides) are also present in small quantities (Lazaridou et al., 2004; Zamora et al., 2006). Sugars are the major components of honey. The total and reducing sugars amounts vary from 63.89% to 86.49%, from 53.38% to 78.29%, respectively. Sucrose contents ranged from 0.45% to 21.66%. According to the Turkish Food Codex Honey Notification (2000) [16], honey can contain up to 5% sucrose. In this research, only six honey samples showed an appropriate sucrose content ranging from 0.45% to 3.49%. Other samples have higher sucrose content than 5%. The sucrose level can increase if the beekeeper overfed the bees with sugar during the spring (Anklam, 1998). Moreover, high content of this sugar means an early harvest of the honey (Azeredo et al., 2003). The sugars content of honey has been reported by many scientists (Ouchemoukh et al., 2005; Tosun & Keleş 2002; Yılmaz & Yavuz, 1999). Honey is rich in sugar and a good source of energy. This is very important in human nutrition, especially for babies, children, and sportsmen, and in situations demanding urgent energy.

The ash content is a quality criterion for honey botanical origin; the honeydew honeys have higher ash content than blossom honeys present (Downey et al., 2005; Ouchemoukh et al., 2005). Honey has a rather low mineral content that varies widely depending on the particular botanical origin, pedoclimatic conditions, and extraction technique (Baumgart et al., 1993). The ash content of the studied honey samples ranged from 0.03% to 0.50%. In the case of honey, clustering based on the mineral composition can distinguish honeys by their geographical or floral provenance.

The acidity of honey is due to the presence of organic acids, particularly the gluconic acid and inorganic ions such as phosphate and chloride. The pH of honey is affected by conditions during extraction and storage, which also affects texture, stability and shelf life (Corbella & Cozzolino, 2006). pH is of great importance during honey extraction and storage due to its influence on texture, presentation, and endurance (Özcan et al., 2006; Terrab et al., 2002). Acidity in honey was calculated as total acidity. The mean value for total acidity was found in the range of 14.61– 53.44 meq/kg. Honeys have a pH in the range of 3.11–4.58. That is, all honeys were acidic. These results agreed with data reported by Iurlina and Fritz (2005), Ouchemoukh et al. (2005), and Özcan et al. (2006). The variation in acidity among different honey types may be attributed to variations in these constituents due to harvest season (de Rodríguez et al., 2004).

5-Hydroxymethylfurfural measurement is used to evaluate the quality of honey. In fresh foods, the HMF level is close to zero (Kus et al., 2005). The HMF content of honey is an indicator of freshness, and it is known that heating of honey results in the formation of HMF, which is produced during acid-catalyzed dehydration of hexoses such as fructose and glucose. Hydroxymethyl furfural (HMF) is one of the most typical products of degradation, and several factors influence the formation of HMF in honey: temperature and time of heating; storage conditions; use of metallic containers, and the chemical properties of honey, which are related to the floral source from which the honey has been extracted, these indicate pH, total acidity, mineral content; however, no information on the correlation between chemical characteristics and HMF level of honey is available (Corbella & Cozzolino, 2006; Downey et al., 2005; Fallico et al., 2004; Özcan et al., 2006; Spano et al., 2006; Zappalà et al., 2005). Its concentration tends to increase as the honey ages, as a function of the low pH values, the botanical origin, the humidity, and from thermal and/or photochemical stress, until it even reaches levels of some tenths of mg/kg (Spano et al., 2006). 5-Hydroxymethylfurfural values showed between 0.77 to 5.76 mg/kg. All samples showed low

HMF content, below the allowable limit of 40 mg/kg (Turkish Food Codex, Honey Notification, 2000).

Honey diastase activity is a quality factor influenced by honey storage and heating, thus indicating honey freshness and overheating (de Rodríguez et al., 2004). The diastase activity of analyzed honey samples was between 0 and 38.5 °G. Eighteen honey samples showed an appropriate diastase number above 8 °G and only two samples showed values below 8 °G, the limit allowed by Turkish Food Codex, Honey Notification (2000). These values are in close agreement with those found by other researchers (Andrade et al., 1999; Costa et al., 1999; Tosun & Keleş, 2002). One of the samples did not show diastase activity. This shows that the sample was synthetically produced. Tosun and Keleş (2002) have determined the diastase activities of some honey samples to be below eight and of one sample to be zero, which is an indication of overheating or imitation.

The color parameters L^*, a^*, and b^* measured using the Minolta colorimeter were within the range of 36.04–57.12 (–1.92)–7.46 and 2.69–22.91, respectively—one of the simplest red component and yellow components. The color of honey is related to the content of phenolics, HMF, pollen, and minerals (Lazaridou et al., 2004).

Microbial Counts

Twenty honey samples obtained from the Erzurum province of Turkey were examined for the possible presence of microorganisms. The total bacteria, fungi, osmophilic yeasts, thermophilic *Bacillus*, and coliform bacteria counts in analyzed samples are shown in Table 2. Table 3 shows the correlation results among microbiological groups. There was a significant correlation between the total bacteria count and fungi and osmophilic yeasts (Table 3).

Table 2.
The Results of the Microbiological Quality of Honey

	Log_{10} Microorganisms/g Honey				
Organisms	***x*[a]**	**Range**	**CV[b]**	***n*[c]**	**%[d]**
Total bacteria	2.02	1.78–2.26	25.14	20	–
Total coliforms	1.00	1.00–1.00	—	20	100
Fungi	1.21	1.00–1.43	37.81	20	55
Osmophilic yeasts	1.18	0.97–1.39	36.68	20	80
Thermophilus *Bacillus* spores	1.00	1.00–1.00	—	20	100

Note: [a]Geometric mean.
[b]Coefficient variation
[c]Number of samples/analyzed
[d]Percent of samples with negative growth.

Table 3.
Correlation Coefficient between the Microorganisms in Honey

Organisms	**Total bacteria**	**Total coliforms**	**Fungi**	**Osmophilic Yeasts**
Total coliforms	a			
Fungi	0.450*	a		
Osmophilic yeasts	0.506*	a	0.867**	
Thermophilus *Bacillus* spores	a	a	a	a

Note: *Correlation is significant at the $p < .05$ level.**Correlation is significant at the $p < .01$ level. [a]Correlation is significant at the $p > .05$ level.

While the least total bacteria count was 1.5 × 10 cfu/g, the highest was determined as 1.3×10^3 cfu/g. The fungi count of honey samples ranged between <10 and 2.6×10^2 cfu/g. The osmophilic yeasts count of honey samples ranged from <10 to 4.0×10^2 cfu/g. In addition, 20% of honey samples contained detectable levels of osmophilic yeasts.

However, fewer than 10 cfu/g were in 80 % of honey samples (Table 2). Variation in levels of thermophilic Bacillus spores among samples has not been observed. Counts of thermophilic Bacillus spores were found below detectable levels in analyzed samples (<10 cfu/g). Coliform bacteria count was also found to be below detectable level in analyzed samples (<10 cfu/g).

The microorganisms of more interest in honey are total bacteria, fungi, total coliforms, osmophilic yeasts, and bacterial spores. Honey is expected to present low numbers and little variety of microorganisms since it possesses antimicrobial properties that discourage growth, besides a low water activity (Snowdon & Cliver, 1996). Total bacteria count provides very general information and is a general indicator of the microbial quality of honey. Total bacteria counts of honey samples can vary from zero to tens of thousands per gram for no apparent reason. Variations in total bacteria numbers may be due to the sample type, the honey's age, the time of harvest, and the analytical technique. The number of total bacteria in the analyzed samples was very low compared with other research results (Table 2). There was also a significant ($p < .05$) correlation between the total bacteria count and fungi and osmophilic yeasts (Table 3). Fathy and Ashour (1997) determined that the total honey count reached 2.0×10^4 cfu/mL. Migdal et al. (2000) found that total count of aerobic bacteria varies from 1.0 × 10 to 4.6×10^5 cfu/g.

As can be seen from Table 2, in the present study, we determined fungi in 9 (45 %) samples, ranging in number from < 10 to 2.6×10^2 cfu/g. There was a significant correlation between fungi and osmophilic yeasts (Table 3). Migdal et al. (2000) obtained similar results, with counts between < 10 and 9.0×10^3 cfu/g. The numbers of osmophilic yeasts were low. The incidence of osmophilic yeasts varied from sample to sample. There was also a significant correlation between osmophilic yeasts and the total bacteria (Table 3). Snowdov and Cliver (1996) consider yeasts to be among the main microorganisms that interfere with the quality of honey. As fermentation is proportional to the concentration of osmophilic yeasts, honey with a very yeast count is not likely to be palatable. Piana et al. (1991) assayed 50 samples of Italian honey and primarily found osmophilic yeasts in the range of 1.0 to 3.5×10^3 cfu/g. Only 34 of the samples contained osmophilic yeasts. An average of nine yeast colonies per gram of honey was found in 35 retail samples tested by Nakano and Sakaguchi (1991).

Thermophilic Bacillus spores were found below detectable levels in 20 honey samples analyzed in this research (Table 2). In a study of the incidence of *Bacillus* and *Clostridium* spores in honey factories and retail outlets, Kokuba et al. (1994) detected no *C. botulinum* but detected spores, most of them of the genus *Bacillus*, in 67 of 71 (97%) samples.

In the present study, none of the 20 samples contained total coliforms, in agreement with the data obtained by Snowdov and Cliver (1996) and Rall et al. (2003) who reported the absence of pathogenic bacteria in honey. Coliform counts are an indicator of hygienic quality. Since fecal contamination of honey has not been

reported, an assay for coliforms could also be used as a general indicator of fecal contamination as well as sanitation.

Based on the results obtained, we concluded that the microbiological properties of the honey samples collected from Erzurum are very good. In this study, incidence of coliform bacteria were determined as low <10 cfu/g, that honeys can have an acceptable quality with coliform bacteria. The results obtained agreed with the requirements of the Turkish Food Codex, Honey Notification, 2000. Eighteen of the studied samples are found to be low in moisture and, therefore, safe from fermentation. Nevertheless, two samples had moisture contents slightly above 20%, likely due to earlier honey extraction from hives. Only six honey samples showed an appropriate sucrose content; other samples had higher sucrose content than 5%. All honeys were acidic. All of the samples showed low HMF content, below the allowable limit of 40 mg/kg (Turkish Food Codex, Honey Notification, 2000). The diastase activity of analyzed honey samples was between 0 and 38.5 °G. Further investigations are required to determine phenolic contents, antioxidant activity, and antimicrobial activity of honey. The results obtained from this study will also be a guide for future studies.

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