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Analysis of some physical, chemical and microbiological aspects of honey samples produced and consumed in Turkey

Análisis de algunos aspectos físicos, químicos y microbiológicos de las muestras de miel producidas y consumidas en Turquía

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ABSTRACT

Analysis of honey produced in the western part of Turkey was carried out in this study. Fifty honey samples, collected from the local market in this region were analyzed for their physicochemical parameters including hydroxymethylfurfural (HMF), electrical conductivity, sucrose, free acidity, moisture and water insoluble impurities, in addition to pathogenic microorganisms present. HMF amounts determined in 47 samples were found to be between 1.9 and 98.0 mg/kg with good reproducibilities (%RSD: 3.2% or better). There were no pathogenic microorganisms in any of the investigated samples. Electrical conductivity that gives information about their mineral contents was measured, and the mean value was found to be 0.33 mS/cm (N=34). In general, most of the samples were considered to meet the requirements of Turkish Food Codex and European Commission Directives.

Keywords

honey • quality • HMF • sucrose • moisture

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RESUMEN

En este estudio se analizó muestras de miel producida en la zona oeste de Turquía. Se trabajó con 50 muestras del mercado local de la región, en las cuales se determinaron parámetros físico-químicos incluyendo el hidroximetilfurfural (HMF), conductividad eléctrica, sacarosa, acidez libre, humedad e impurezas insolubles en agua además de microorganismos patógenos presentes. Las cantidades de HMF determinadas en 47 muestras oscilaron entre 1,9 y 98,0 mg/kg con buena desviación estándar de la reproducibilidad (% de RSD/DSR 3,2% o mejor). No se encontraron microorganismos patógenos en ninguna de las muestras analizadas. La conductividad eléctrica, que aporta información sobre el contenido mineral, resulta en un promedio de 0,33 mS/cm (N=34). En general se encontró que la mayoría de las muestras cumple los requerimientos del Codex alimentario turco y de las directivas de la Comisión Europea.

Keywords

miel • calidad • HMF • sacarosa • humedad

INTRODUCTION

Honey is known as the first and most reliable sweetener which has been used by human beings until now. High nutritional value and the fast absorption of its carbohydrates upon consumption are some of the main characteristics of honey (11, 32). It was found that honey has antimicrobial properties in addition to some positive health effects in healing wounds and burns, and in cancer care (3, 9, 16).

Floral origins utilized by bees and climatic conditions are the main factors affecting its composition and properties. Honey mainly consists of water, sugars (glucose, fructose, sucrose, maltose, and higher sugars), gluconic acid, lactone, nitrogenous compounds, minerals, and some vitamins (21).

Hydroxymethylfurfural (HMF) content is the parameter used for the evaluation of honey freshness and/or overheating.

The European regulation allows a maximum HMF content of 40 mg/kg (10).

In general, HMF is determined in honey samples during quality control, but HMF

does not provide any information about botanical and/or geographical origin (2).

Electrical conductivity, closely related to the concentration of mineral salts, organic acids, and proteins, is another parameter in quality control, as well as for the discrimination of honeys. Measurement of honey electrical conductivity is an effective tool to ascertain its mineral contents. In literature, many studies can be found on this topic (1, 33). It has been shown that free acidity in honey is an indication of its organic acid content and this parameter is useful for the evaluation of honey fermentation (19). Another criterion for honey quality is its content of simple carbohydrates (sugars).

The sugar content of honey is about 95% dry matter, mainly in the form of monosaccharides. Glucose and fructose are the predominant sugars in honey. Sucrose is in low amounts because it is converted to glucose and fructose in the process of honey maturing (8).

The water content of natural honey samples is an indicator of the degree of maturity and storage method. Hence, moisture determinations have long been done to assess the quality of natural honey samples (5).

The main purpose of this study was to analyze honey samples for their physico-chemical parameters to figure out whether they are in conformity with the Turkish Food Codex and European Commission Directives in terms of hydroxymethylfurfural, electrical conductivity, sucrose, free acidity, moisture, water insoluble impurities and pathogenic microorganisms.

MATERIALS AND METHODS

Chemicals

Only analytical reagent grade chemicals were used throughout this study. Pure water, obtained from a Nüve NS 112 water purification system, was used in all sample and standard preparation processes. All working standards and solutions were prepared daily and stored in the refrigerator.

Methods

All of the parameters investigated were determined according to procedures listed in Turkish Standards (TS).

TS 3036 standard (26, 31) was used for the determination of hydroxymethylfurfural, sucrose, free acidity, moisture and water-insoluble impurities, and TS 13366 standard (30) was used for the electrical conductivity measurements.

Each sample was analyzed 3 times for the calculation of both the mean value and standard deviation.

A Nova 60 UV-VIS instrument was used for the determination of hydroxymethylfurfural. For each HMF measurement, 10.0 g of honey was used. Para-toluidine (100 g/L)

and barbituric acid (0.5%) were applied for the color development. All of the measurements were performed at 550 nm.

Intensities measured were used to calculate the HMF amounts in the samples.

Electrical conductivity of the honey samples was measured with a TetraCon 325 model LF 330 conductivity meter.

Sucrose cannot react with the Fehling reagents, but the other reducing sugars give positive reactions. In the case of heating at 65-67°C and with the help of conc. HCl, sucrose splits into its monosaccharides, and then it readily reacts with the Fehling reagents positively. Hence, HCl was added to honey samples and the mixture was heated to 65-67°C for the conversion.

Fehling A solution (hydrated copper (II) sulfate in water) and Fehling B solution (potassium sodium tartrate and sodium hydroxide in water) were used for the determination of sucrose. Reaction with Fehling reagents using 2-3 drops of methylene blue with continuous boiling gave a brick-red precipitate.

The volumes of honey solution used to obtain the brick-red precipitate before and after inversion were used for the calculation of sucrose in honey (26, 31).

For the moisture determinations, a Macnimpex Abbe Refractometer was used. This method is based on the measurement of the refraction extent as light passes through a sample. The amount of refraction is related to the amount of solid and fluid in the honey sample. All measurements were done at 20°C. Refractive index as measured by refractometry was converted to %moisture using the conversion table given in TS 3036 standard (26, 31).

Free acidity of the honey samples was measured by titration with 0.10 M NaOH using phenolphthalein as indicator.

For the determination of water-insoluble impurities, 20.0 g honey was mixed with distilled water set to 80°C.

The mixture was filtered by Gooch crucible and the crucible was washed several times with hot water at 80°C. The crucible was dried at 135°C until no difference was observed between 2 consecutive weight measurements (26, 31).

In the determination of pathogenic microorganisms, procedures prescribed in Turkish Standards were used: TS 12812 for *Clostridium botulinum* (25), TS EN ISO 16654 for *E.coli* O157:H7 (27), TS EN ISO 6579 for *Salmonella* spp. (28), TS 6582-2 for *Staphylococcus aureus* (24), TS EN ISO 7937 for *Clostridium perfringens* (29), and TS EN ISO 11290-1 for *Listeria monocytogenes* (23).

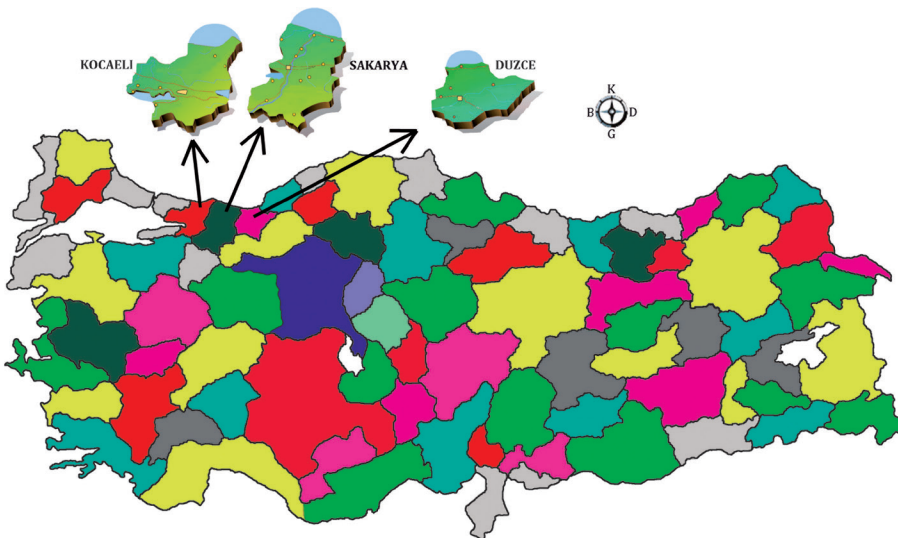
Sample collection and storage

Fifty honey samples were obtained from local markets in the western part of Turkey (Kocaeli, Sakarya and Düzce).

Although these honey samples were supplied in this region, the origin of the honey samples analyzed is from different types of wild flowers on mountain highlands in different parts of Turkey.

All the samples taken and transferred to the laboratory were kept at 2-4°C until analysis. Samples were analyzed within a week for hydroxymethylfurfural, electrical conductivity, sucrose, free acidity, moisture, and water-insoluble impurities to avoid any possibility of decomposition. A map showing the sample origins is shown in figure 1.

In the figure 1, the western cities Kocaeli, Sakarya and Düzce, and their towns can be seen. Total surface area and population of this region are 11122 m² and 2771198, respectively.



Source / Fuente: <http://www.csb.gov.tr>

Figure 1. Sample points in Turkey.

Figura 1. Puntos de muestreo en Turquía.

RESULTS AND DISCUSSION

The results for hydroxymethylfurfural, electrical conductivity, sucrose, free acidity, moisture, and water-insoluble impurities are given in table 1 (page 268). Each sample was analyzed at least 3 times and mean value of the results is reported. Free acidity, humidity and water insoluble impurities were determined in all samples while HMF, electrical conductivity and sucrose were determined in 47, 34 and 49 samples, respectively.

Hydroxymethylfurfural

HMF is a parameter used for honey quality (2). It can be seen in table 1 (page 268), that 47 honey samples were analyzed for their HMF contents. Concentrations of HMF were found to be in the range of 1.9-98.0 mg/kg, with a mean value of 25.64 mg/kg.

In the Turkish Food Codex (TFC) and the European Union Directive (EUD), the maximum limit for HMF is given as 40 mg/kg.

In this study, 7 honey samples were found to be of unacceptable quality based on the HMF limits given in TFC and EUD (10, 22).

Significantly higher values which are well above the legal limit might be due to the heat-treatments of samples.

In literature, there are many studies on the HMF content of different honey samples. In one study, the physicochemical parameters of honey samples taken from Marmara region and East Anatolia of Turkey are provided and the HMF amount were 31.8 ± 1.20 and 30.5 ± 1.38 mg/kg in honey samples from these 2 regions, respectively (15). Gulfranz *et al.* (2010) analyzed 40 samples of different honey types collected from Pakistan and concentrations of HMF were found to be 30.5 ± 0.49 mg/kg. The HMF results were comparable with those above (14).

In another study, determinations of HMF in 11 types of honey sold in Porto Alegre were made and it was observed that HMF amounts were in the range of 0.191 to 6.206 mg/kg (6).

In another study performed in Argentina (one of the World's largest producers), the physico-chemical properties of honey produced in the province of Chaco were analyzed for the contribution to the characterization of these honeys and HMF amount was found between 2.3-24.5 mg/kg (20).

Electrical conductivity

Electrical conductivity of honey samples was measured to obtain information about their mineral contents.

The mean value was found to be 0.33 mS/cm (N=34). Conductivity of samples was measured and the results were in the range of 0.01 to 0.76 mS/cm.

In TFC and EUD, the allowable maximum limit for this parameter is 0.80 mS/cm (10, 22). It is seen in table 1 (page 268), that all of the honey samples analyzed met the TFC and EUD criteria. Similar results were found by Feas (2010) and others who investigated some properties of artisanal honey samples ($n = 45$) collected from the northwest of Portugal.

Electrical conductivity was found in the range of 0.46 to 0.94 mS/cm, with mean value calculated as 0.66 mS/cm.

In another study, Gomes *et al.* (2010), analyzed 5 commercial honey samples taken from Portuguese markets for their floral origins, physicochemical parameters including electrical conductivity, and microbial safety. It was found that electrical conductivity ranged from 0.19 to 0.53 mS/cm (13).

Table 1. Results for honey samples interested (number of replicates for each sample is 3).**Table 1.** Resultados para las muestras de miel de interés (el número de repeticiones para cada muestra es 3).

	HMF amount. mg/kg, %RSD: 3.2% or better	Electrical conductivity. mS/cm %RSD: 0.5% or better	Sucrose. g/100g %RSD: 6.2% or better	Free acidity. meq/kg %RSD: 1.4% or better	Humidity %RSD: 0.9% or better	Water insoluble impurities. mg/100 g %RSD: 5.7% or better
Codex Limits (TFC, 2005, EUD, 2002)	40 (Max.)	0.8 (Max.)	5.0 (Max.)	50 (Max.)	20% (Max.)	100 (Max.)
Sample 1	79.1	0.11	3.46	9.8	16.6	70
Sample 2	36.2	0.32	3.80	5.7	18.0	95
Sample 3	12.7	0.42	2.90	25.2	17.2	80
Sample 4	86.7	0.10	0.78	1.5	16.4	82
Sample 5	8.4	0.70	1.38	16.2	16.6	27
Sample 6	7.8	0.10	2.60	2.7	17.8	15
Sample 7	13.8	0.24	3.29	4.4	17.8	37
Sample 8	80.9	0.51	2.08	7.7	17.4	40
Sample 9	79.6	0.66	2.11	5.4	17.4	45
Sample 10	-	0.69	3.36	3.8	16.6	57
Sample 11	-	0.21	2.97	4.0	18.2	55
Sample 12	8.0	0.34	4.40	3.5	17.6	75
Sample 13	36.0	0.18	3.50	3.8	16.4	77
Sample 14	66.3	0.76	0.51	17.7	15.4	72
Sample 15	9.1	0.59	0.50	3.9	16.8	70
Sample 16	2.3	0.15	4.85	9.5	16.8	70
Sample 17	5.9	0.11	4.04	7.0	17.0	<10
Sample 18	30.4	0.17	4.69	5.8	18.6	79
Sample 19	30.9	0.50	3.80	3.8	19.4	70
Sample 20	30.9	0.43	2.23	18.0	18.2	59
Sample 21	6.1	0.23	0.92	14.3	16.6	10
Sample 22	50.5	0.02	0.61	2.6	17.8	46
Sample 23	19.1	0.44	2.20	9.3	16.6	42
Sample 24	23.8	0.68	4.59	20.9	18.4	60
Sample 25	4.5	0.40	3.76	2.8	19.4	80
Sample 26	8.2	0.43	3.02	2.2	19.0	23
Sample 27	4.1	0.30	3.60	3.9	19.7	80
Sample 28	11.8	0.01	4.46	33.2	19.8	62
Sample 29	3.8	0.23	3.46	4.1	16.8	23
Sample 30	98.0	-	3.40	5.8	14.8	90
Sample 31	37.0	-	4.00	14.1	14.8	50
Sample 32	37.7	-	3.40	12.2	16.4	50
Sample 33	18.7	-	4.10	3.7	17.8	87
Sample 34	31.6	-	4.10	2.4	16.4	52
Sample 35	12.5	-	4.30	4.1	16.6	80
Sample 36	11.7	0.46	3.05	4.1	16.5	72
Sample 37	3.4	0.23	3.20	6.7	16.8	60
Sample 38	3.0	0.30	4.20	4.4	17.8	61
Sample 39	1.9	0.04	4.20	1.7	18.4	62
Sample 40	15.8	0.10	3.31	3.1	17.2	32
Sample 41	17.2	-	4.12	13.7	17.0	75
Sample 42	35.0	-	3.90	1.3	13.8	40
Sample 43	-	-	2.03	20.4	17.4	74
Sample 44	16.3	-	4.07	15.8	16.3	73
Sample 45	27.7	-	3.73	4.1	17.4	25
Sample 46	12.0	-	4.07	13.6	16.3	74
Sample 47	22.8	-	3.75	24.9	17.6	74
Sample 48	14.0	-	4.36	13.7	16.4	42
Sample 49	16.2	-	2.80	16.7	16.4	59
Sample 50	15.8	-	-	2.4	16.4	83

Sucrose

In literature, it is stated that the sucrose amount in honey is an important parameter to detect heavy sugar feeding of the bees or adulteration by direct addition of sucrose (17). In the Turkish Food Codex and the European Union Directives, maximum allowable limit for sucrose in honey is 5.0 g/100 g (10, 22).

In the study, concentration range for sucrose was found to be between 0.50 and 4.85 g/100 g (N=49), with the mean value of 3.22 g/100 g. In literature, many scientists have reported on sucrose for the characterization and/or quality control of honey samples.

Devillers *et al.* (2004), analyzed 469 samples of fir, cinder heather, chestnut, lavender, acacia, rape, and sunflower honey for moisture, conductivity, diastase activity, pH, free acidity, color, hydroxymethylfurfural, and percentages of fructose, glucose, sucrose, erlose, raffinose, and melezitose and found that sucrose concentrations were in the range of 0-5.3%, with a mean of 0.742% (7).

It is clear that our mean value was much higher than that.

Mendes *et al.* (1998), analyzed 25 different honey samples taken from Portuguese markets and they found that the mean percentage of sucrose for 2 samples was above the allowable limit of 5.0%, while in our study none of the honey samples showed sucrose values above 5.0 g/100 g (17).

Free acidity

Free acidity is the indication of the organic acid content of honey and it is an efficient tool for the evaluation of honey fermentation (19).

According to TFC and EUD, 50.0 meq/kg of free acidity is the maximum allowable limit for honey (10, 22).

In this study, the contents of free acidity were found to be between 1.3 and

33.2 meq/kg and the mean value was calculated as 8.83 meq/kg. It is seen in table 1 (page 268), that all of the honeys met TFC and EUD specifications in terms of free acidity.

Similar results are shown in the literature. Rodriguez *et al.* (2010) and others characterized the citrus blossom honey of Sierra Morena by melissopalynological and physicochemical analyses. It was found that free acidity ranged from 7.0 to 26.3 meq/L (18).

In another study, Forcone *et al.* (2009) performed the palynological and physicochemical characterization of honeys from the northwest area of Santa Cruz (Argentinean Patagonia). They found the free acidity to range between 9 and 20 meq/kg (12).

Moisture

Moisture content is another indication for the quality of honey. Crystallization in certain types of honey can be accelerated by high moisture content. In addition, high moisture can increase water activity to values where certain yeasts could grow (13). Good-quality honey has a low water content.

Honey is likely to ferment and lose its freshness and unpasteurized honey may show wild yeast growth if the water content is greater than 19%.

The moisture of raw honey can be as low as 14% and honey containing up to 20% of water is not recommended for mead-making (4).

The maximum limit for moisture set by TFC and EUD is 20.0% (10, 22).

In this investigation, the mean value for moisture was found to be 17.2% and values varied between 13.8 and 19.8%. None of the samples exceeded the legal limit of 20.0%.

This results were similar to those published by Gomes *et al.* (2010) with 15.9 to 17.2% in their study (13).

A range for moisture contents of honey was found to be 16.8-18.6% by Feas *et al.* (2010).

Water-insoluble impurities

One of the parameters to decide whether honey is of good quality is the amount of water-insoluble impurities. Visually clean and clear honeys can be categorized to be of good quality.

Contaminants include particles of wax, parts of bees, splinters of wood, and dust certainly make honey look unappetizing. Therefore, such honey is of low quality (4).

In this study, water-insoluble impurities in samples were in the range of 10 to 95 mg/100 g, except for sample 17 which had a value lower than 10 mg/100 g.

The mean value for this parameter was calculated to be 58.5 mg/100 g. In TFC and EUD, the legal maximum limit for this parameter is 100 mg/100 g. In this study, all samples met the legal requirements set by TFC and EUD (10, 22).

Microbiological analysis

Microbiological analysis was also performed in this study on the quality of honey samples. Pathogenic microorganisms, namely, *Clostridium botulinum*, *E.coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens* and *Listeria monocytogenes* were determined due to their categorization as health-impairing microorganisms.

All of the samples were collected via sterile containers and stored at 2-8°C before the analysis. According to the Turkish Food Codex, there must be no pathogenic microorganisms present (22).

All of the analyses were performed in sterile media and it was observed that there were no live cells in any of the samples investigated.

CONCLUSION

Fifty honey samples taken from the western part of Turkey were analyzed and 86% of the samples reached the quality parameters and met national honey specifications and European Union Directives. It was observed that physicochemical properties showed variations among honey samples.

Results found for electrical conductivity, sucrose, free acidity, humidity and water insoluble impurities were within the imposed legal limits of the Turkish Food Codex and European Union Directives.

HMF amounts did not fall within the maximum limits in seven honey samples. There were no pathogenic microorganisms found in the analyzed samples. Moisture content as an indication for the quality of honey due to acceleration of crystallization with high moisture content was determined in all samples and values were found to be lower than 20.0% which is the maximum limit set by TFC and EUD.

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