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Physicochemical Properties of Honey Produced at Different Altitudes

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ABSTRACT

The types and densities of honeyed plants vary depending on the altitude. This causes the amount and Physicochemical structure of honey produced in apiaries of different altitudes to change. In this study, honey harvest from the honeybee colonies placed at different altitudes in the same geographical region was carried out in the first week of September. Standard laboratory methods were used to determine some physicochemical properties of the honey samples. Some of the minimum and maximum average values obtained as a result of the analysis of honey samples; moisture 14.70% and 18.60%, free acidity 20.50 meq/kg and 25.30 meq/kg, pH 3.20 and 4.30, EC 0.22 and 0.44 mS/cm, fructose 32% to 45%, glucose 0.10% to 0.18%, sucrose from 0.66% 1.80%, maltose ranged from 0.66% to 1.80%. Also, HMF ranged from 1.80 mg/kg to 3.50 mg/kg, proline 530.00 mg/kg and 710.00 mg/kg, Density from 1.44 g/cm^3 to 1.49 g/cm3, Invertase 20.30 U/kg-28.50 U/kg, Diastase activity 13.23-19.07 and Total phenolic content ranged from 76.00-94.00 g. It has been determined that the physicochemical structures of honey produced at different heights are statistically different from each other. This study aims to determine the effect of altitude difference on the quantity and physicochemical structure of honey.

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Introduction

Honey is the sweet substance that honey bees collect the nectar they secrete from the flowers or living parts other than flowers (leaves, branches, or stems) and mature by adding their enzymes. Honeybees also make use of the excretions of some insects that live and feed on plants to make honey. Nectars, which are collected and mixed with enzymes by honey bees, are stored in honeycombs and mature (Mendes et al., 1998).

Honey is a very important source of energy and is used as an ingredient in the production of many foods due to its taste, color, aroma, odor, and viscosity (De RodríGuez et al., 2004). It has also been used as a component of traditional medicines from past to present due to its antibacterial, anti-inflammatory, therapeutic, and anti-tumor properties (Oršolić et al., 2005; Pichichero et al., 2009; Swellam et al., 2003). Honey is an economically very important beekeeping product that is produced by honeybee colonies. Honey contains about 80% carbohydrates (35% glucose, 40% fructose, and 5% sucrose) and 20% water. It also contains more than 180 substances, including amino acids, vitamins, minerals, enzymes, organic acids, phenolic compounds (Cengiz et al., 2018). Its pH is about 4.0 (Ouchemoukh et al., 2007; White Jr, 1980). Honey contains some amino acids, the most important of which is proline (Serra Bonvehi and Escolà Jordà, 1997). The amount of proline is used to determine the maturity of honey and whether it is fake or not. The most important enzymes in honey are invertase, diastase (amylase), and glucose oxidase (Krell, 1996). These enzymes, secreted by the worker bees and added to the nectar, enable the nectar to turn into honey. Hydroxymethylfurfural (HMF) is rarely found in fresh and unheated honey. HMF is formed as a result of the degradation of sugars in honey by the effect of heat (Belitz et al., 1992).

The quantity and characteristics of honey vary according to the flora of the region where the bee yard is located, the season, environmental factors, and the applications of the beekeeper (Erdogan et al., 2009; Erdoğan, 2019; Kaškonienė et al., 2010; Leite et al., 2000).

Table 1. The locations and altitude of apiaries.

Apiaries	Locations of apiaries	Altitude (m)
1	40° 37'48.73"N-41° 11'0.71"E	900
2	40° 38'8.55"N-41° 10'29.84"E	1000
3	40° 38'27.27"N-41° 9'34.88"E	1100
4	40° 39'6.50"N-41° 8'29.00"E	1200
5	40° 39'40.39"N-41° 7'58.97"E	1300
6	40° 40'7.51"N- 41° 7'51.26"E	1400
7	40° 40'35.22"N-41° 7'38.28"E	1500
8	40° 41'8.16"N-41° 7'41.94"E	1600
9	40° 42'0.17"N-41° 7'21.34"E	1700
10	40° 42'48.81"N-41° 6'51.03"E	1800
11	40° 43'22.36"N-41° 6'52.91"E	1900
12	40° 43'48.10"N-41° 6'55.81"E	2000
13	40° 44'11.74"N-41° 7'22.89"E.	2100

Fructose, glucose amount, fructose/glucose ratio, and glucose/water ratio in the structure of honey are important factors related to the quality of honey. Besides, the fructose/glucose ratio also indicates the ability of honey to crystallize (Kaškonienė et al., 2010; Manikis and Thrasivoulou, 2001; White Jr, 1980). The low moisture content of honey prevents its fermentation (Akhtar et al., 2014).

This study aimed to determine the physicochemical properties of honey produced in apiaries with the same hive types and growing conditions and different altitudes.

Material and Methods

Study Area

The study was conducted in a valley at the Kaçkar Mountains in the Caucasian ecological zone which is considered by the World Wild Fund for Nature and by Conservation International as a biodiversity hotspot (Erdoğan and Erdoğan, 2014).

This valley has a rich plant diversity and is a region preferred by stationary and migratory beekeepers. Kaçkar Mountains and Çoruh basin have an important ecological richness in terms of a wide variety of plants, wildlife, and biodiversity (Çakmakçı et al., 2009).

The study was carried out along a streambed that originates from the Kaçkar mountains and joins the Çoruh River. The locations where apiaries are located are given in Table 1.

Care has been taken to ensure a minimum distance of 2000m between the two apiaries. Apiaries were established with a height difference of 100 m from each other. Thirteen apiaries were established within the scope of the study (Figure 1).

The first apiary was established at an altitude of 900m and the 13th apiary at an altitude of 2100m. Ten wooden beehives were placed in each apiary. All of the hives where the colonies are placed are Langstroth-type hives and these hives are painted with linseed oil. Colonies were fed 1/1 sugar syrup until 15 May and prepared for the main nectar flow period. The queen bee of each hive was a Caucasian hybrid and an elder. At the beginning of June, each colony was equated to 10 frames, with eight sealed brood areas. Beehives are covered with an electric fence to protect the colonies from bears and other bee pests.

Harvesting Honey

Honey harvest was done on the same day in all hives at the end of the production season. The harvest was made only from supers. The numbers of the hives from which they were taken were written on the Frames taken from the Honey Holds. After extraction, the honey yield of each hive was determined separately (Carbonari et al., 2016).

Collection of Honey Samples

Honey harvest was carried out in 13 different apiaries on 5 September. Honey harvested from the beehives in each apiary was mixed and three samples were taken from this honey mixture. As a result, 39 honey samples were taken from 13 different apiaries.

Honey samples were placed into 250 ml sterilized glass sample bottles and kept at 18°C until analysis.

Physicochemical Analyses of Honey Samples

Moisture Content

The moisture content of honey was determined according to its refractive index. The thermostat of the refractometer was set to 20°C, and its calibration was made with distilled water (Bogdanov.S, 2009).

Free Acidity and pH

The acidity and pH of honey were determined according to (Ihc, 2009). 10 g of sample was dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker.

The pH was measured with a pH meter and the solution was titrated to pH 8.3 with 0.1 N sodium hydroxide solution. The acidity of honey represents the content of all free acids and is expressed in meq/kg honey.

Electrical Conductivity (EC)

Electrical conductivity EC was measured using a conductivity meter (Hanna Instruments, Mauritius) and a 20% (W/V) honey solution suspended in milli-Q water (Bogdanov et al., 1997).

Sugar analysis

Sugar analyzes in honey were carried out according to the methods recommended by the International Honey Commission (IHC) (Ihc, 2009).

To determine the sugar composition of honey, 5 g of honey sample was weighed and dissolved in distilled water. After honey dissolved, 25 ml of methanol was added and it was completed to 100 ml. The prepared solution was filtered through a 0.45 syringe filter and read with a RID detector in an HPLC device (Thermo Scientific UltiMate 3000; Thermo Scientific Amino Gold column).

Hydroxymethyl Furfural (HMF)

HMF amount in honey was determined using sodium bisulfite and Carrez I and Carrez II solution (Ihc, 2009). The absorbance of the sample solution against the reference solution at 284 and 336 nm was determined in quartz cuvette by using a spectrophotometer (Hach Lange DR6000 UV–VIS Spectrophotometer, Germany). The results obtained are expressed in mg/kg.

Density

To determine the density of honey was calculated by using the pycnometer method and using the formula below.

$$D=\frac{(W2\text{-}W1)g}{Wml}$$

In this equation;

(W1) = the mass of the empty pycnometer

(W2) = the mass of the full pycnometer.

The honey samples were heated at 50°C for 30 minutes to make filling in the pycnometer (James et al., 2009).

Table 1. The result of physicochemical analyses of honey samples produced at different altitudes

Samples	Honey yield (Kg/colony)	Moisture (%)	pH	EC[(1)] (mS/cm)	Free Acidity (meq/kg)	Maltoz (%)	Glikoz (%)	Fruktoz (%)	Sükroz (%)
1	15.30^{bc}	16.80^{e}	3.70^{c}	0.28^{b}	21.70^{b}	1.14^{f}	20.00^{bc}	32.00^{a}	0.16^{f}
2	15.00^{b}	15.50^{c}	3.90^{d}	0.32^{d}	22.50^{c}	0.90^{d}	23.00^{ef}	33.00^{a}	0.10^{a}
3	16.73^{cd}	17.40^{g}	3.20^{a}	0.44^{h}	20.60^{a}	1.20^{g}	22.00^{de}	35.00^{b}	0.12^{bc}
4	17.00^{de}	16.30^{d}	3.40^{b}	0.22^{a}	23.60^{e}	1.50^{h}	21.00^{cd}	37.00^{c}	0.15^{ef}
5	17.20^{de}	18.40^{h}	3.30^{ab}	0.35^{e}	25.80^{ı}	0.66^{a}	20.00^{bc}	39.00^{d}	0.12^{bc}
6	18.03^{ef}	15.50^{c}	4.20^{fg}	0.34^{e}	24.20^{f}	0.87^{c}	19.00^{ab}	33.00^{a}	0.18^{g}
7	17.53^{def}	17.20^{f}	4.00^{de}	0.22^{a}	26.40^{i}	0.69^{b}	22.00^{de}	37.00^{c}	0.15^{ef}
8	18.93^{f}	16.30^{d}	3.60^{c}	0.34^{e}	20.50^{a}	1.20^{g}	24.00^{fg}	39.00^{d}	0.14^{de}
9	21.50^{g}	18.60^{ı}	3.70^{c}	0.34^{e}	22.80^{d}	1.10^{e}	23.00^{ef}	42.00^{e}	0.15^{ef}
10	17.47^{def}	15.20^{b}	4.10^{ef}	0.37^{f}	24.40^{g}	1.60^{ı}	21.00^{cd}	40.00^{d}	0.10^{a}
11	16.37^{bcd}	14.80^{a}	4.30^{g}	0.39^{g}	22.40^{c}	1.50^{h}	25.00^{g}	44.00^{fg}	0.11^{ab}
12	15.33^{bc}	14.70^{a}	3.60^{c}	0.30^{c}	25.30^{h}	1.80^{i}	19.00^{ab}	43.00^{ef}	0.14^{de}
13	13.27^{a}	16.40^{d}	3.40^{b}	0.31^{cd}	22.50^{c}	0.67^{a}	18.00^{a}	45.00^{g}	0.13^{cd}

[(1)]EC, Electrical conductivity

Table 2**.** The result of physicochemical analyses of honey samples produced at different altitudes.

Samples	Total antioxidant	HMF[(1)]	Prolin	Density	İnvertase	Diastase Unite	TPC [(2)]	TFC [(3)]
				(g/cm^3)	(U/kg)	(DN)	(mg GAE /100 g)	(mg CE/100 g)
1	320.00^{a}	3.50^{j}	580.00^{d}	1.46^{bc}	20.30^{a}	15.20^{bcd}	90.00^{f}	40.00^{a}
2	380.00^{f}	2.70^{g}	670.00^{j}	1.48^{de}	25.50^{f}	16.13^{bcde}	87.00^{d}	45.00^{c}
3	379.00^{f}	2.10^{c}	640.00^{h}	1.44^{a}	23.70^{d}	15.06^{abcd}	94.00^{g}	47.00^{d}
4	355.00^{b}	2.20^{d}	590.00^{f}	1.46^{bc}	28.50^{l}	14.20^{abc}	88.00^{de}	55.00^{e}
5	386.00^{h}	1.90^{b}	570.00^{c}	1.47^{cd}	25.40^{e}	17.27^{efg}	79.00^{b}	43.00^{b}
6	369.00^{d}	2.90^{h}	550.00^{b}	1.48^{de}	26.30^{ı}	16.20^{cde}	85.00^{c}	59.00^{f}
7	377.00^{e}	3.10^{ı}	530.00^{a}	1.49^{e}	22.40^{b}	17.07^{def}	87.00^{d}	55.00^{e}
8	384.00^{g}	2.39^{e}	530.00^{a}	1.44^{a}	25.80^{g}	15.23^{bcd}	93.00^{g}	48.00^{d}
9	370.00^{d}	2.50^{f}	588.00^{e}	1.45^{ab}	25.90^{h}	18.23^{fg}	89.00^{ef}	45.00^{c}
10	370.00^{d}	2.10^{c}	650.00^{ı}	1.47^{cd}	22.50^{c}	19.07^{g}	76.00^{a}	42.00^{b}
11	360.00^{c}	1.80^{a}	630.00^{g}	1.46^{bc}	27.80^{j}	16.13^{bcde}	93.00^{g}	40.00^{a}
12	390.00^{ı}	2.31^{e}	710.00^{l}	1.45^{ab}	27.90^{k}	13.23^{a}	90.00^{f}	58.00^{f}
13	385.00^{gh}	2.11^{c}	680.00^{k}	1.47^{cd}	25.80^{g}	14.17^{ab}	94.00^{g}	64.00^{g}

[(1)] HMF. Hydroxymethylfurfural. [(2)] TPC. Total phenolic content. [(3)] TFC. Total flavonoid content.

Invertase and Diastase Activity

The invertase enzyme in honey was measured according to the method reported by (Bogdanov.S. 2009). The diastasis number of honey harvested from researched beehives was determined using UV/VIS Spectrophotometer according to the method suggested by IHC (International Honey Commission) (Ihc. 2002).

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of honey produced in the study area (Ferreira et al., 2009). The results obtained are expressed as mg gallic acid equivalent (GAE) per 100 g of honey.

Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used with minor modifications to determine the total flavonoid content (TFC) of honey produced as a result of the study (Gomes et al., 2010).

The results obtained because of the analysis are expressed as mg catechin equivalent (CE) per 100 g of honey.

Amino Acid Content

The 200 mg honey sample was put into the hydrolysis tube. It was closed by adding 5 ml of 6 N HCl on it. This tube was then incubated at 110°C for 24 hours. The solution from the incubator was filtered with Whatman No 1 filter paper. 200 µl of the filtrate was evaporated for one hour at 140°C. To the sample whose moisture had been evaporated. pH 2.2 and 1.0 ml 0.12 N buffer (11.8g Trisodium citrate dehydrate. 6.0 g citric acid. 14 ml thiodiglycol. 12 ml 32% HCl and 2 g phenol dissolved in one liter of distilled water) were added. The amino acid content of the samples was determined following the manufacturer's instructions (Sycam amino acid analyzer. S433-Sycam-Germany) (Mohammed and Babiker. 2010).

Statistical Analyses

ANOVA (IBM SPSS 22 Statistics software) statistical package program was used to compare the average physicochemical parameters of honey samples. The significance level was taken as P>0.05 in the analyzes. Tukey's HSD post hoc test was used to compare the means of the results.

Results and Discussion

Honey Yield

In terms of honey yield. the highest average value was obtained from the 9th apiary with 21.50 kg/colony and the lowest average value was obtained from the 2nd apiary with 15.00 kg/colony (Table 1). In the experiment. the amount of honey produced in honeybee colonies placed at different altitudes was found to be statistically different from each other (P>0.05). The height of the area where bee colonies are placed is a very effective factor in the quantity and quality of honey produced.

Table 3. The amino acid content of honey is produced at different altitudes (mg/kg).

Samples	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Lysine
1	1767.80^{c}	417.46^{g}	1444.05^{a}	854.05^{c}	594.70^{f}	191.17^{b}	817.30^{d}
2	1626.30^{a}	446.65^{h}	1501.75^{b}	785.75^{a}	618.70^{h}	206.00^{d}	746.00^{a}
3	1697.18^{b}	354.95^{b}	1536.55^{c}	864.47^{d}	535.24^{b}	178.03^{a}	802.23^{b}
4	1862.58^{g}	367.95^{d}	1922.85^{h}	997.54^{h}	526.07^{a}	236.10^{i}	1733.10^{h}
5	1788.12^{e}	360.55^{c}	1884.40^{g}	947.64^{g}	562.85^{c}	231.37^{ı}	1869.23^{ı}
6	1770.20^{d}	335.43^{a}	2134.50^{ı}	1176.96^{i}	615.95^{g}	208.13^{e}	1629.10^{g}
7	2201.80^{l}	541.50^{l}	2555.80^{i}	1219.86^{j}	813.50^{k}	232.30^{ı}	2304.07^{k}
8	2179.68^{k}	503.45^{k}	2615.03^{j}	1342.52^{k}	888.53^{l}	209.00^{e}	2479.10^{l}
9	2113.58^{j}	459.47^{i}	2837.07^{k}	1438.80^{l}	732.27^{j}	216.00^{g}	2165.30^{j}
10	1979.85^{ı}	451.52^{ı}	1617.10^{d}	922.50^{e}	641.40^{ı}	212.23^{f}	883.10^{f}
11	1821.55^{f}	459.47^{j}	1682.13^{e}	848.65^{b}	667.87^{i}	228.27^{h}	805.233^{c}
12	1900.69^{h}	383.25^{e}	1721.50^{f}	933.87^{f}	578.005^{e}	197.27^{c}	867.00^{e}
13	2086.17^{i}	397.47^{f}	1077.40^{ı}	1077.40^{ı}	568.10^{d}	262.23^{j}	1872.00^{i}
Samples	Valin	Methionine	Tryptophan	Phenylalanine	İsoluecine	Leucine	Sarcosine
1	168.17^{b}	691.23^{bc}	391.20^{a}	848.10^{c}	495.27^{a}	1124.27^{c}	1414.10^{a}
2	166.27^{a}	643.07^{ab}	400.20^{b}	933.07^{h}	542.20^{d}	1012.10^{a}	1571.07^{e}
3	173.17^{c}	591.27^{a}	428.23^{d}	914.17^{f}	515.17^{b}	1052.27^{b}	1445.17^{b}
4	333.07^{g}	784.17^{def}	619.00^{gı}	792.30^{b}	752.30^{ı}	1377.23^{f}	2165.00^{ı}
5	366.17^{ı}	858.17^{fg}	557.03^{g}	723.10^{a}	662.17^{h}	1530.20^{h}	2121.23^{h}
6	433.03^{i}	717.30^{bcd}	581.27^{h}	896.00^{e}	624.30^{g}	1667.00^{i}	1796.30^{g}
7	443.07^{j}	875.10^{g}	821.17^{k}	1133.10^{k}	940.27^{l}	1511.30^{g}	2913.27^{l}
8	460.30^{k}	805.10^{efg}	879.00^{l}	1110.23^{j}	893.23^{k}	1572.13^{ı}	2680.23^{k}
9	576.03^{l}	1024.03^{h}	772.07^{j}	1280.23^{l}	780.30^{i}	1829.13^{j}	2418.17^{j}
10	182.00^{e}	767.23^{cde}	422.30^{c}	916.07^{g}	535.17^{c}	1248.20^{e}	1527.13^{c}
11	180.07^{d}	714.00^{bcd}	432.23^{e}	1007.27^{i}	586.00^{f}	1123.23^{c}	1696.30^{f}
12	187.13^{f}	656.30^{ab}	463.00^{f}	987.20^{ı}	556.23^{e}	1168.20^{d}	1561.07^{d}
13	359.27^{h}	870.27^{fg}	668.17^{i}	856.13^{d}	813.03^{j}	1529.10^{h}	2338.07^{ii}

Moisture Content

The moisture content of honey samples collected from apiaries in the study area varies between 14.70% and 18.60%. The highest humidity was found in honey samples taken from the ninth apiary (18.60%) and the lowest moisture level was found in honey samples taken from the 12th apiary (14.70%) (Table 1).

In this study. it was found that the difference between honey produced at different altitudes in terms of moisture content was statistically significant (P>0.05). The climatic conditions of the region where honey is harvested and the period when it is harvested affect the moisture content of the honey. When honey is harvested before it is ripe. its moisture content is high (Finola et al., 2007). Moisture is an important parameter used to detect honey maturity. According to the Turkish Food Codex Honey Communique (2020/7). the water content of honey should be below 20% except for (*Erica sp.* and *Calluna Vulgaris*) honey which can have a maximum moisture value of 23%.

In a study. it was determined that the moisture content of honey produced in high altitude regions is lower than honey produced at sea level (Batu et al., 2013). The results were similar with some studies (Şahinler et al., 2001; Yılmaz and Küfrevioğlu. 2001) and lower than some of them (Şahinler and Gül. 2004). The high moisture content of honey causes it to ferment in a short time. Therefore. honey with low humidity has a much longer shelf life (Fredes and Montenegro. 2006).

Acidity and pH

The free acidity values of the honey produced because of the study vary between 20.50 meq/kg and 25.30 meq/kg (Table 1). The pH values of the honey samples obtained from the study fields vary between 3.20 and 4.30 (Table 1). It was determined that there is a statistically significant difference between honey produced at different elevations in terms of pH and acidity values (P>0.05) (Table 1).

The acidic structure of honey consists of organic acids such as gluconic acid. The acidic feature of honey gives it its unique taste and ensures its microbial stability (Faustino et al., 2015).

However, if the honey is not harvested at the right time and its moisture content is high. or if it is stored in unsuitable environments. its acidity may increase too much. The values we have obtained are lower than the maximum 50 meq/kg permitted by international regulations (Commission. 1981)

The results obtained regarding the acidity of honey are similar to the values obtained from some previous studies (Fallico et al., 2004; Yılmaz and Küfrevioğlu. 2001). The results obtained are greater than the value reported by Russo-Almeida (1997) but smaller than the value reported by Sunay (2006). As a result of the studies conducted. the differences in the acidity values of honey are caused by the harvest times and honeyed plants (Küçük et al., 2007).

The pH of honey is highly affected by extraction and storage conditions. which affects its heritage and shelf life? Most bacteria can grow in neutral and slightly alkaline environments. while yeasts and molds can grow in acidic environments. Therefore. the pH of honey is very important for bacterial and fungal growth (Conti. 2000).

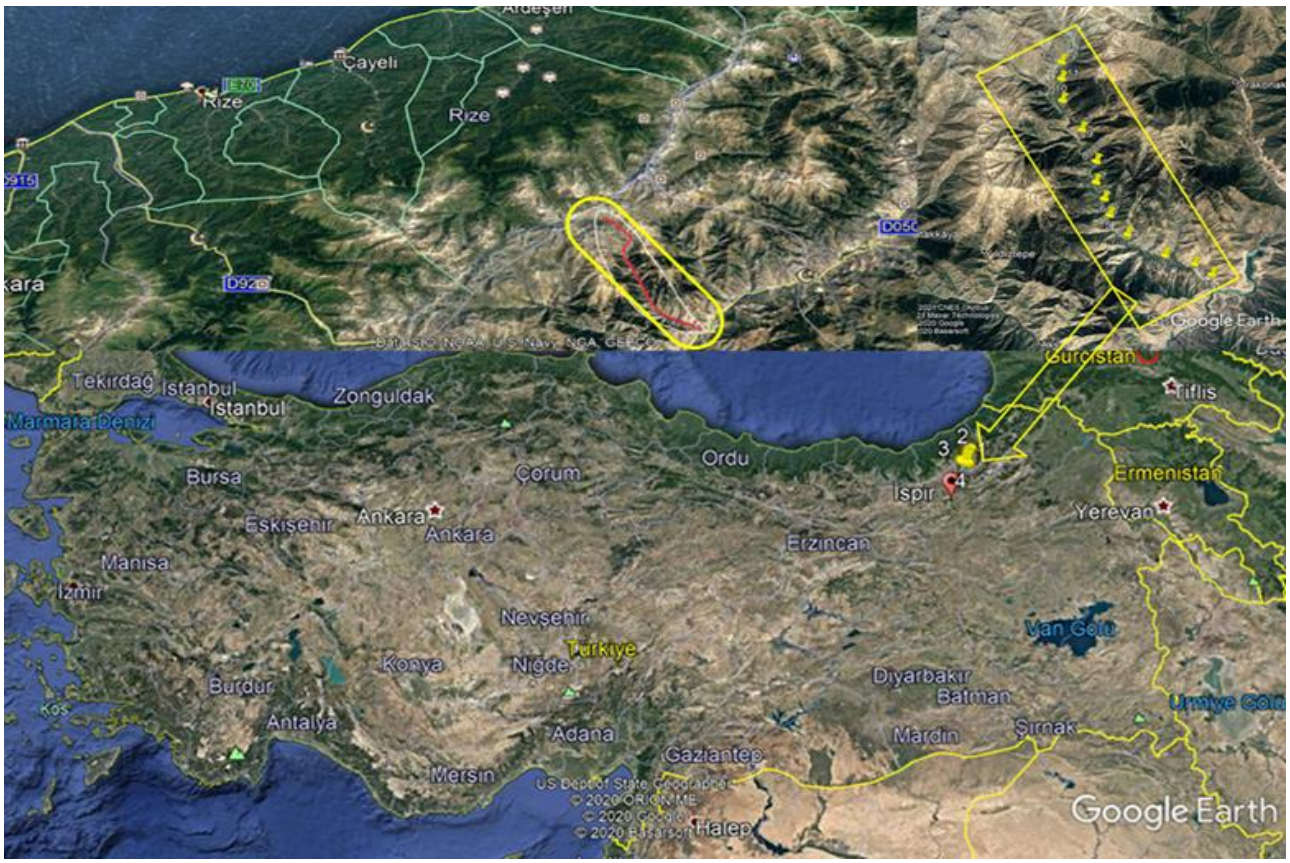

Figure 1. The geographical location of apiaries.

Some sources reported that the pH value of honey should be between 3.2 and 4.5 (Bogdanov.S. 2009). The values we obtained were found close to the values obtained in previous studies (Draiaia et al., 2014).

Electrical Conductivity (EC)

Electrical conductivity values of honey samples vary between 0.22 and 0.44 mS/cm. The values of honey samples belonging to different altitudes in terms of electrical conductivity values are statistically different (P>0.05) (Table 1).

The electrical conductivity of honey varies according to the density of mineral salts. organic acids. and proteins. Their density varies depending on the plant from which the nectar is taken. This feature can also be used to detect plants that are the source of honey (Terrab et al., 2002). Therefore. EC is frequently checked in routine honey controls. In a study. it was reported that honey produced from plants such as Acacia (*Robinia pseudoacacia*). lavender (*Lavandula* sp.). and milkvetch (*Astragalus* sp.) Showed lower conductivity (Can et al., 2015). The values we determined were similar to some other studies (Ashraf and Akram. 2008; Şahinler et al., 2001).

According to the Turkish Food Codex Honey Notification and European Union standards. the electrical conductivity in honey can be a maximum of 0.8 mS/cm. except for honey obtained from some plants (*Arbutus unedo. Erica spp. Eucalyptus camaldulensis. Tilia spp. Calluna vulgaris. Leptospermum. Melaleuca spp.*).

Average EC values of honey samples obtained from the study area are below the maximum honey limit (0.8 mS/cm).

Sugar Analysis

Average values of sugar contents such as fructose. glucose. sucrose. and maltose of honey collected from the study area are given in (Table 1). The average values of honey samples ranged from 32% to 45% fructose. 18% to 25% glucose. 0.10% to 0.18% sucrose. 0.66% to 1.80% maltose.

Statistically significant differences were found in glucose ratios of honey samples (P>0.05) (Table 1). The amount of sucrose detected in this study ranged from %0.10 to %0.18.

Statistically significant differences were found between honey samples (P>0.05) in terms of sucrose and maltose contents (Table 1). Sugars constitute 95% of the dry matter contained in honey (Dag et al., 2006). Glucose and fructose. which are mono-saccharides. are the main sugars in honey (Mendes et al., 1998). The sugar content of honey varies according to the season. vegetation. altitude. geographic conditions. and climate (Anklam. 1998; Da Silva et al., 2016). These values obtained as a result of the analysis are higher than 60/100 g. which is accepted as the minimum value by the CAC Honey Standard. TFC Honey Communique. and EU directive of 2001/110/EC (Cac. 2001; Union. 2001). The results we obtained were lower than (Vit et al., 2009) close to the values reported by (Kahraman et al., 2010) and higher than the values reported by (Estevinho et al., 2012).

Sucrose and maltose are sugars found in honey in very small amounts. Sucrose. which comes from nature. is converted into glucose and fructose by the enzyme invertase (Azeredo et al., 2003). For this reason, there is very little sucrose in honey stored under appropriate conditions.

The amount of sucrose detected in all of the samples is below the legal limit of 5%. The amount of sucrose determined as a result of the analysis is much lower than the values reported by (Ünal and Küplülü. 2006).

However, the amount of sucrose determined was close to the values reported by (Küçük et al., 2007).

The detected maltose value was higher than that reported by (Habib et al., 2014) and lower than that of (Manzanares et al., 2017).

Hydroxymethyl Furfural (HMF)

The HMF content detected in honey samples varies between 1.80 mg/kg and 3.50 mg/kg. Statistically significant differences were found between the HMF amounts of honey samples (P>0.05) (Table 2). The presence of HMF amount is an indicator of the freshness of the honey. HMF is not found in fresh and unheated honey. The amount of HMF in honey varies depending on the heating. storage conditions. pH. and the flowers that are the source of the honey (Fallico et al., 2004).

HMF is formed by the decomposition of fructose in honey because of unsuitable storage or heating for a long time. The high HMF content in honey indicates that the honey is heated for a long time or mixed with processed sugar (Gebremariam and Brhane. 2014). The HMF values obtained because of the analysis of honey are far below the maximum value of 40 mg/kg reported by the WHO/FAO Codex. TFC. and EU.

The values we obtained because of the analysis of the samples were lower than the average values (3.3. 0.71. and 4.95 mg/kg) stated because of previous studies (Sunay. 2006; Yılmaz and Küfrevioğlu. 2001).

The results obtained are consistent with the value below 10 mg/kg reported by Gül (2008).

Density

The average density of the honey samples obtained because of the experiment varies between 1.44 g/cm^3 and 1.49 g/cm^3. Significant differences were found between the averages of the density of honey samples (P>0.05) (Table 2). These values were found very close to the values (1.3-1.51 g/cm3) obtained in a previous study conducted in Nigeria (Lullah-Deh et al., 2018) (Table 2).

Invertase

The invertase content of honey samples ranges between 20.30 U/kg and 28.50 U/kg (Table 2.) Significant differences were observed between honey samples in terms of invertase activity (P>0.05) (Table 2). Invertase is a natural enzyme used to determine the freshness of honey. The difference between the averages of the invertase values of the honey samples obtained is thought to be due to the height of the study areas and the plants that are the source of the nectar.

According to the values obtained as a result of the analysis. it was determined that all samples (40 units'/kg honey) complied with the standard reported by (Commission. 1981).

Diastase Activity

The number of diastases in honey samples ranges from 13.23 to 19.07 (Table 2). As a result of the analysis. it was revealed that the difference between the averages in terms of the number of diastases of the honey samples was significant (P>0.05).

Diastase enzyme is a natural enzyme found in honey. This enzyme plays an important role in honey's biological value. The diastase activity in honey varies according to the sucrose ratio in the nectar. the nectar flow rate. the flower that is the source of the nectar. and the age of the bees (Özcan et al., 2006). Exposure of honey to high temperatures or long storage inactivates diastase (Ünal and Küplülü. 2006).

The values obtained because of the analysis. this value was above the 8 limit values determined by FAO/WHO Codex. TFC. and EU. The number of diastases in honey was higher than 10.31 reported by (Şahinler and Gül. 2004) and 11.58 by (Ünal and Küplülü. 2006). However, as a result of a study conducted. it was lower than the values reported for Eucalyptus (33.9) and Chestnut (27.3) honey (Fallico et al., 2004)

Total Phenolic Content (TPC)

The values of the total phenolic content (TPC) obtained as a result of the analysis of honey samples varied between 76.00 and 94.00 (mg GAE/100 g). As a result of the analysis of variance (ANOVA). it was revealed that the difference between the mean phenolic content of honey samples was statistically significant (P>0.05) (Table 2).

The phenolic content of honey comes from the plants that are the source of honey. As a result of the study. the results obtained in the previous studies (47-98 mg GAE/100 g honey) (Saxena et al., 2010). (18.730-107.213 mg GAE/100 g honey) (Isla et al., 2011). (32.59-114.75 mg GAE/100 g honey) (Meda et al., 2005). higher than minimums and lower than maximums.

Total Flavonoid Content (TFC)

The average values obtained because of the analysis of the total flavonoid content (TFC) of honey are given in (Table 2).

Average TFC values obtained in honey varied between 40.00 and 64.00 (mg CE/100 g). The differences between these mean values were found to be statistically significant (P>0.05). Flavonoids are low molecular weight phenolic compounds related to the aroma and antioxidant capacity of honey. These results have been reported as a result of previous studies (Boussaid et al., 2018) (22.45 mg CE/kg honey). rosemary (16.24 mg CE/kg honey). thyme (14.77 mg CE/kg honey). orange (11.12 mg CE kg honey) and horehound (11.02 mg CE/kg honey mg CE/kg honey) are higher than the values.

Amino Acid Content

Average values of the amino acid content of honey produced at different altitudes are given in Table 3. Three amino acids (Theanine Arginine and Alanine) were detected in the highest concentrations in the honey obtained in the study. Of these amino acids. Thiamine and Arginine were detected in the highest amount in honey produced from the ninth apiary and Alanine from the 8th apiary. As a result of the analysis of variance. it was revealed that there were significant differences between the amino acids of honey samples (P>0.05) (Table 3).

Some studies have reported that the most dominant amino acid in honey is proline (Iglesias et al., 2004; Truzzi

et al., 2014). Amino acids. especially proline. are used to determine the maturity of honey and in some cases as an indicator of the adulteration of honey. Because of the analysis of honey samples. Proline values were determined between 530.00 mg/kg and 710.00 mg/kg. These values are above the minimum value reported by IHC. 180 mg/kg (Ihc. 2009). The source of proline amino acid is the secretions that honey bees add to the nectar during the collection and transportation of nectar. Proline values determined in honey samples was higher than reported by Aazza et al., (2013) (453.09 mg/kg). Gonçalves et al., (2018). (566.6 mg/kg). Hermosin et al., (2003). (490.3 mg/kg). by Belay et al., (2017). (470.6 mg/kg) and Serrano et al., (2004). (112.08 mg/kg). Also. as a result of the studies. it is lower than the 180 mg/kg reported by Habib et al., (2014). With 1044.36 mg/kg reported by (Aazza et al., 2013).

Proline value for IHC European heather honey has been reported to be between 309 and 1033 mg/kg (Beekman. 2005) Although the amino acid contents in honey vary according to the plants that are the source of honey. 180 mg proline kg^{-1} was accepted as the minimum value (Bogdanov.S. 2009). According to this criterion. all honey had a value above the minimum limit.

Conclusions

One of the most influential factors on the physicochemical structure of honey is honeyed plants that form the structure of honey and are the source of nectar. Densities and types of plants with honey vary depending on altitude.

To determine the effects of this variety. the physicochemical structures of honey produced in the same geographic region. in the same valley. under the same conditions but at different altitudes were examined. Because of the analysis. it has been determined that all samples comply with the GSO standards. Because of the analysis. it was determined that honey belonging to each altitude is statistically different from the other. This study reveals the changes in the chemical structure of honey depending on height. By looking at these results. beekeepers can determine the height at which to position their apiaries.

Because of this study. the highest honey yield was obtained in the apiary with a height of 1700 m. This situation shows that honeyed plants at 1700m altitude secrete a much greater amount of nectar and humidity and temperature inside and outside the hive keep the honey consumption of the worker bees at a minimum.

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