BIOCHEMICAL ANALYSIS OF BITLIS-HIZAN (TURKEY) REGION'S HONEY

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Abstract. In this study, biochemical analyses of honey produced in Hizan district of Bitlis province which is one of the important honey centers of Turkey, were performed. In the research, 7 honey samples collected from villagers dealing with beekeeping in different regions of Hizan were studied. Hydroxymethylfurfural (HMF), antioxidant activities, sugar, flavonoid and total phenolic levels were measured with the HPLC device. As a result, the flavonoid, sugar and total phenolic levels of 1, 4, 5 and 7 honeys were similar to the properties of natural honeys. The HMF values of all honey samples were found to be in accordance with the value determined in the Turkish Food Codex Honey Communiqué. It was determined that the honeys used in our study have scavenging properties on DPPH and ABTS radicals. In addition, the maltose value of the 3 and 5 honey samples was found to be slightly higher than that of the other honey samples. Since maltose is formed as a result of the hydrolysis of starch, it cannot be found naturally in plant juices. Honey samples with high maltose content are considered to be produced under unnatural conditions. The antioxidant and chemical properties of Bitlis-Hizan honey make them high value-added quality products. As a result, it was seen that the results comply with EU standards and the Turkish Food Codex Honey Communiqué.
Keywords: *ABTS, Bitlis (Hizan) honey, DPPH, HMF, phenolic compounds*

Introduction

Honey is a product obtained form honeycombs, where it is made by bees through the collection of nectar. It consists of approximately 200 different components. Honey is rich in flavonoids, phenolic acids, vitamins, amino acids, minerals, organic acids, and enzymes. It is easily digestible and possesses nutritious, anti-inflammatory, and therapeutic qualities. Serving various purposes, honey is a multifunctional food source (Ozmen and Alkin, 2006; Spilioti et al., 2014; Taş-Küçükaydın et al., 2023). The physical properties and chemical content of honey vary depending on geographical and botanical factors.

Honey primarily contains 82% carbohydrates, 17% water, 0.7% various minerals, 0.3% protein, along with some vitamins, phenolic compounds, organic acids, and amino acids as free micro and macro components. Beekeeping is a significant agricultural and livestock activity practiced worldwide, with a history dating back to ancient times. Given that approximately 3/4 of the world's flower species are found in Turkey, it is possible to say that high-quality honey is produced in the country (Kayral, 2005; Küçükaydın et al., 2023).

Bitlis is one of the important regions for honey production, especially in Mutki and Hizan districts. The physical structure and vegetation of these areas make them ideal for beekeeping. The richness of forested areas in Hizan, with plant varieties such as thyme,

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clover, mint, alfalfa, pennyroyal, sage, watercress, hogweed, thistle, hawthorn, cherry, apple, pear, pomegranate blossom, pistachio flower, and balsam, highlights the prominence of beekeeping as a significant source of livelihood for the region.

Bee products, including honey, propolis, beeswax, royal jelly, and pollen, are increasingly recognized for their benefits to humanity. The demand for bee products continues to rise due to these advantages. This study aims to conduct biochemical analyses of honeys produced in Hizan, Bitlis, one of the important honey centers in the country. The study reveals the nutritional quality, antioxidant effect, and compliance with the Turkish Food Codex of the region's honeys.

Material and methods

Honey samples and location

In the summer months of the year 2020, honey samples were collected from seven different locations in the Hizan region of Bitlis province in Turkey (*Fig. 1; Table 1*). All honey samples were preserved according to storage procedures to prevent contamination.

Determination of flavonoid contents

For the evaluation of flavonoid quantities, a chromatographic analysis was conducted using a reversed-phase column with a 5 μm inner diameter, PREVAIL C18 (15 × 4.6 mm). The mobile phase consisted of a combination of methanol, water, and acetonitrile in a 46/46/8, v/v/v ratio, containing 1% acetic acid. Prior to use, the mobile phase was filtered through a 0.45 μm membrane and degassed using an ultrasonic apparatus. Following the RP-HPLC separation, Diode Array Detector (DAD) assessed these flavonoids using miricetin, rutin, quercetin, and morin at wavelengths of 280 nm and 254 nm for naringin and catechin. The injection volume was set at 10 μL, and the flow rate was adjusted to 1.0 ml/min. Comparison of reaction times and UV spectra of standard references was employed to validate the chromatographic peaks of the study. The quantity was measured using both peak integration and standard methods. All chromatographic procedures were conducted at a temperature of 25°C (Zu et al., 2006).

Sugar analysis

A homogeneous mixture was obtained by mixing 10 g of honey samples with distilled water for each group, with a repetition number of n = 5. The liquid component was then removed, and the volume of the entire filtrate was calculated. Subsequently, it was examined using an HPLC apparatus connected to a UV detector. The mobile phase used in the experiment was a combination of acetonitrile + water (v/v) in a ratio of 75/25%. For analysis, a Supelcosil NH2 column (250 × 4.6 mm, 5 μm) with a wavelength of 190 nm was employed. The sugar content was determined using an external standard method (Camara et al., 1996).

Free radical scavenging activity (DPPH)

The DPPH or free radical scavenging activity was measured using the Brand-Williams method (Brand-Williams et al., 1995). DPPH (2,2-diphenyl-1-picrylhydrazyl) was generated at a concentration of 25 mg/l and used as a free radical in methanol. Test

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tubes were filled sequentially with 25, 50, 100, 200, and 400 µl of honey extract and 3.9 ml of DPPH solution. Prepared for each sample with a repetition number of n = 5. The mixtures were incubated in a dark room at room temperature for 30 min, and at the end of the incubation, absorbances were measured at 517 nm using a spectrophotometer against a blank.

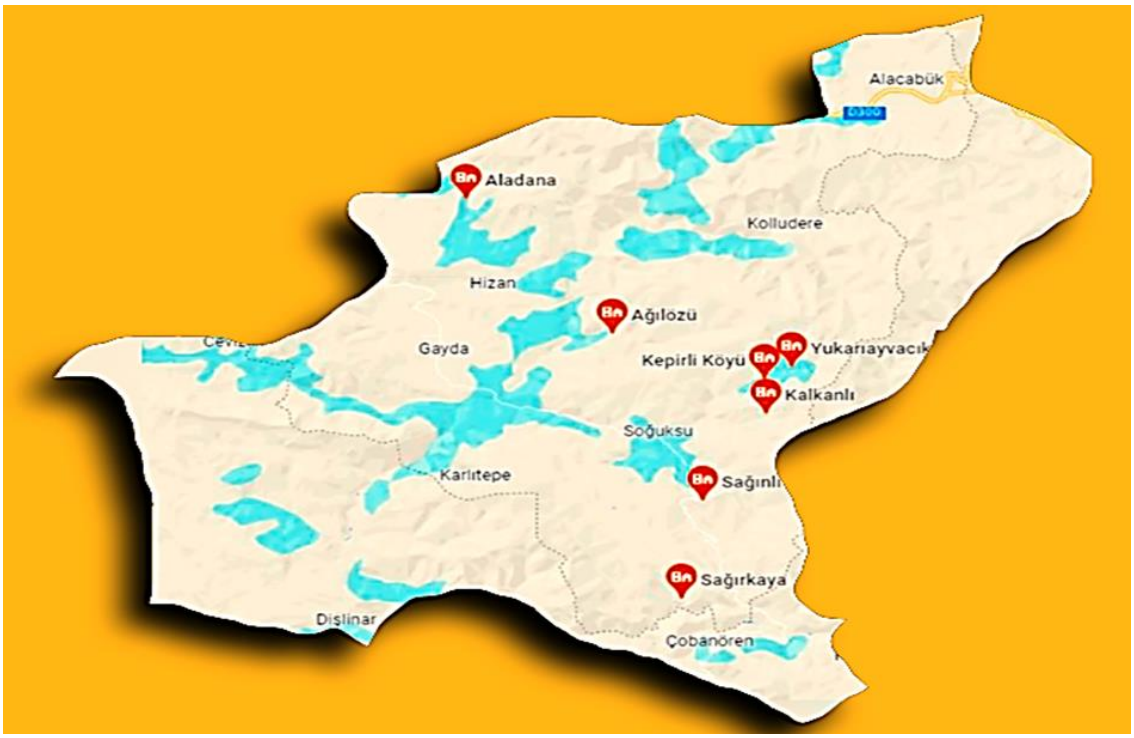

Figure 1. *Locations of the collected honey samples on the map (Google maps, 2022)*

Table 1. *Coordinates of the villages where honey samples were taken, altitude and harvest time of honey*

Sample No.	Village	Coordinate	Elevation	Honey collection time
1	Aladana Village	N:38.307, E:42.412	1788 m	July 2020
2	Ağılözü Village	N:38.193, E:42.518	1596 m	August 2020
3	Kalkanlı Village	N:38.131, E:42.635	2044 m	August 2020
4	Kepirli Village (Nurs)	N:38.158, E:42.632	2135 m	August 2020
5	Yukarıayvacık Village	N:38.169, E:42.656	2109 m	August 2020
6	Sağınlı Village	N:38.068, E:42.587	1552 m	July 2020
7	Sağırkaya Village	N:37.995, E:42.571	1581 m	August 2020

The remaining amount of DPPH was estimated as the free radical scavenging activity based on the decrease in absorbance. The following formula was used to calculate the results (Brand-Williams et al., 1995):

$$\% = (\text{ControlABS} - \text{SampleABS}) / \text{ControlABS} \times 100$$

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ABTS• + radical scavenging activity test

After preparing the radical solution (ABTS), 4 mL was poured into test tubes. Subsequently, 25, 50, 100, 200, and 400 mL of honey extract (n = 5) were added sequentially, and the mixture was incubated in the dark at room temperature for 2 h. The absorbance of the samples was measured at 734 nm against a PBS (Phosphate Buffer, pH = 7.4) blank. The amount of eliminated ABTS radicals from the medium is directly proportional to the decrease in absorbance (Re et al., 1999). The following formula was used for the ABTS radicals scavenged by the extracts:

$$\% \text{ ABTS Scavenging Activity} = [(A0-A1) / A0] \times 100$$

A0: Absorbance value of the control; A1: Absorbance value after applying the extract (Re et al., 1999).

Total phenolic determination

To determine the total phenolic compound content in the collected honey samples, the Folin-Ciocalteu reagent was used following the method described by Singleton (1999). For each group, 1 ml was taken from the ethyl alcohol extraction of 10 g of honey (with a repetition number of n = 5), and 1 ml of Folin-Ciocalteu reagent was added. The mixture was vortexed, and after 3 min, 3 ml of 2% Na_2CO_3 solution was added. The mixture was left at room temperature for 2 h. After the specified times, the density of the samples was measured at 760 nm against distilled water. This technique was repeated three times for each honey sample. Quercetin and pyrocatechol were used as reference phenolic chemicals. To create the calibration curve, 25 mg of quercetin and 25 mg of pyrocatechol were dissolved in separate test tubes, and concentrations and absorbances were calculated. The calibration curve was drawn, and calculations were made after taking 0, 50, 100, 200, and 400 μL.

Hydroxymethylfurfural (HMF) derivative analysis

Honey samples were analyzed for HMF using an HPLC device, following the method outlined by Zappal et al. (2005). For each group, 10 g of honey (n = 5) was dissolved in a 10 ml water/methanol mixture (%90/%10, v/v) in 50 ml Falcon tubes. The solution was centrifuged at 5000 rpm at + 4°C for 5 min, and 1 ml was transferred to vials using an automatic sample collector. HPLC apparatus was utilized for the analysis, with the mobile phase consisting of a combination of water and methanol (%90/%10, v/v). The column used was a Supelcosil Ascentis RP Amide HPLC column (150 × 4.6, 5 μm). A DAD detector was employed with a wavelength of 283 nm selected for the HMF study.

Prolin analysis

The proline content of honey samples was determined using a spectrophotometric technique (Cinar, 2010). For each group, 5 g of honey (n = 5) was weighed into a 100 mL volumetric flask, and it was dissolved with distilled water before completing to 100 mL. To each tube, 1 mL of 3% ninhydrin solution, 1 mL of formic acid, 0.5 mL of honey solution, 0.5 mL of proline standard solution (0.8 mg/25 mL), and 0.5 mL of distilled water (blank) were added. Using a multi-vortex mixer (with the tube caps

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closed), the mixture was rapidly stirred for 15 min. After being kept in a boiling water bath for 15 min, homogeneous tubes were placed in a 70°C water bath for an additional 10 min. To each tube removed from the water bath, a combination of 5 mL (50%/50%) of 2-propanol and water was added. After 45 min, the absorbance of the tubes was measured using a spectrophotometer with a 10 mm light path and a wavelength of 510 nm.

The proline amount in honey was calculated using the following formula:

$$\text{Prolin (mg kg-}^{1}\text{)} = Es/Ea \times E1/E2 \times 80$$

Es: Sample solution absorbance, Ea: Proline standard solution absorbance (average of two readings), E1: Proline concentration of standard solution in milligrams, E2: honey solution in g, 80: The dilution factor is represented by this number (Cinar, 2010).

Statistical analysis

For statistical analyses, the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software package was used. The LSD test was employed for comparisons among groups. Results were presented as mean ± SEM (Standard Error of the Mean). The level of statistical significance was considered with respect to p values, and $p < 0.05$ was accepted.

Results

Honey samples' ABTS radical and DPPH free radical scavenging effects

According to the results of ABTS radical scavenging, it was determined that the 1st and 6th honey samples at a concentration of 25 µL exhibited a difference statistically significant radical scavenging effect compared to other honey samples, while the 5th honey sample showed the lowest level of this effect. At concentrations of 50 µL and 100 µL, the 1st and 4th honey samples, at 200 µL, the 4th and 7th honey samples, and at 400 µL, the 3rd, 4th, and 7th honey samples showed a more pronounced radical scavenging effect compared to other samples at the specified concentrations. A parallelism in the radical scavenging properties was observed for each group concerning the increase in concentration. Radical scavenging activity was found to be low at low concentrations and higher at high concentrations (*Table 2*).

Table 2. *Antioxidant enzyme activities in honey samples*

Honey samples	ABTS (25 µL)	ABTS (50 µL)	ABTS (100 µL)	ABTS (200 µL)	ABTS (400 µL)
Honey 1	32.333 ± 3.09	46.68 ± 0.88	47.156 ± 0.63[d]	48.893 ± 2.98	64.606 ± 1.71[d]
Honey 2	14.316 ± 1.5	25.476 ± 0.64	45.596 ± 3.91	53.460 ± 0.53	63.313 ± 1.99
Honey 3	19.583 ± 1.26	30.92 ± 0.23	46.316 ± 0.57	63.126 ± 0.65	67.130 ± 0.88
Honey 4	26.456 ± 3.65	39.106 ± 0.99	61.280 ± 0.99	70.356 ± 1.86	72.69 ± 0.96
Honey 5	8.53 ± 1.25	24.033 ± 0.88	40.080 ± 0.08	52.196 ± 2.24	52.203 ± 1.61
Honey 6	36.17 ± 6.80	32.643 ± 0.37	42.716 ± 0.60	57.24 ± 0.62	64.766 ± 2.25
Honey 7	20.26 ± 1.36	35.566 ± 0.54	50.816 ± 0.96	67.51 ± 0.81	68.073 ± 1.49
Routin	80.533 ± 0.17	88.060 ± 0.03	88.433 ± 0.21	89.520 ± 0.31	90.200 ± 0.02

d: $p < 0.001$

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DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging effect results

According to the results of DPPH free radical scavenging, it was observed that the 3rd honey sample exhibited a lower level of antioxidant activity compared to other honey samples at a concentration of 25 µL. At a concentration of 50 µL, the 7th honey sample showed a certain degree of radical scavenging effect compared to other honey samples. At a concentration of 100 µL, the 1st and 7th honey samples had a significant radical scavenging property than other honey samples. At a concentration of 200 µL, again, the 1st and 7th honey samples, and at a concentration of 400 µL, especially the 1st, 7th, and particularly the 3rd honey samples, exhibited trend statistically significant of free radical scavenging activity (*Table 3*).

Table 3. *DPPH radical scavenging activity of honey samples (%)*

Honey samples	DPPH (25 µL)	DPPH (50 µL)	DPPH (100 µL)	DPPH (200 µL)	DPPH (400 µL)
Honey 1	1.640 ± 0.08	4.946 ± 0.14	11.436 ± 0.05	21.940 ± 0.41	37.536 ± 0.89
Honey 2	2.980 ± 0.32	3.113 ± 0.29	5.396 ± 0.26	11.740 ± 1.20	22.346 ± 0.84
Honey 3	0.510 ± 0.01	1.880 ± 0.15	7.650 ± 0.15	18.443 ± 0.15	53.846 ± 0.62
Honey 4	2.093 ± 0.15	1.813 ± 0.38	6.433 ± 0.70	15.276 ± 0.21	25.916 ± 0.96
Honey 5	1.830 ± 0.04	1.650 ± 0.05	5.503 ± 0.80	15.210 ± 0.23	26.230 ± 0.23
Honey 6	3.793 ± 0.20	4.260 ± 0.27	7.510 ± 0.32	14.093 ± 0.26	25.450 ± 1.10
Honey 7	3.226 ± 0.47	10.176 ± 1.31	19.053 ± 0.21	37.333 ± 0.24	39.106 ± 0.39
Routin	70.490 ± 0.20	80.126 ± 0.06	81.650 ± 0.28	84.403 ± 0.24	84.986 ± 0.06

Sugar contents in honey samples

When examined for fructose content, it was observed that the 5th and 7th groups had much higher concentrations of this sugar. The fructose amount in the 1st honey sample was found to be at the lowest level. No statistically significant difference was detected in other groups. When comparing the glucose values of honey samples, again, it was determined that certain amounts of glucose were present in the 5th and 7th samples, while the 1st honey sample had a low level of glucose, and no statistically significant difference was observed compared to other groups. When comparing the sucrose content in honeys, it was found that the sucrose ratio in groups 1, 2, and 5 was difference significantly compared to other groups (*Table 4*).

Table 4. *Sugar levels of honey (µg g-¹)*

Honey samples	(Fructose)	(Glucose)	(Sucrose)	(Maltose)
Honey 1	4.364 ± 0.86^{cd}	4.076 ± 0.25^{cd}	1.556 ± 0.34	5.054 ± 1.05
Honey 2	82.032 ± 0.11	80.586 ± 0.58	1.400 ± 0.25	4.676 ± 0.45
Honey 3	82.964 ± 9.51	83.886 ± 9.24	0.348 ± 0.07^{cd}	6.128 ± 0.74
Honey 4	79.690 ± 8.34	80.218 ± 8.41	0.708 ± 0.12	2.766 ± 0.53^{cd}
Honey 5	114.328 ± 0.21	112.428 ± 0.52	1.400 ± 0.28	6.254 ± 0.08
Honey 6	75.554 ± 5.66	77.290 ± 4.37	0.784 ± 0.02	4.768 ± 0.73
Honey 7	115.184 ± 1.29	108.176 ± 2.24	0.720 ± 0.22	5.620 ± 0.65

cd: $p < 0.0001$

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HMF, prolin, and total phenolic levels

In this study, the average HMF (Hydroxymethylfurfural) values in honey samples varied between approximately 1.893 ± 0.03 μg g^{-1} and 13.663 ± 0.88 μg g^{-1}, with an overall mean of 4.33 μg g^{-1}. The highest HMF value was observed in honey sample 1, while the lowest was found in sample 6. Samples 2, 3, 4, 5, and 7 had similar HMF values (*Table 5*). According to the Turkish Food Codex Honey Regulation, HMF should not exceed 40 mg kg^{-1}. Within this context, it can be concluded that the HMF values of the studied honeys comply with the regulation.

Prolin content serves as a significant criterion for assessing the purity and maturity level of honey. When examining the proline levels among honey samples, it was observed that almost the same amount of proline was present in all samples. The lowest level was calculated for samples 2 and 6, while the highest level was found in sample 4 (*Table 5*). Evaluating the total phenolic content of honey samples revealed lower phenolic levels in all samples except sample 1, with varying but detectable amounts of phenolic substances in other honey samples (*Table 5*).

When assessing honeys based on total flavonoid content, samples 1, 4, and 7 exhibited trend statistically significant amounts of flavonoids compared to other groups. Sample 7 had the highest flavonoid content at 15.353 μg g^{-1}. The lowest amount was observed in sample 2 at 1.050 μg g^{-1} (*Table 5*).

Table 5. *HMF, proline, phenolic and flavonoid substance levels of honey*

Honey samples	HMF (μg g-¹)	Proline (mg kg-¹)	Phenolic (μg g-¹)	Flavonoid (μg g-¹)
Honey 1	13.663 ± 0.88^{cd}	0.254 ± 0.01	2.203 ± 0.02	7.416 ± 0.16^{d}
Honey 2	3.266 ± 0.03	0.202 ± 0.0008	1.243 ± 0.01	1.050 ± 0.26
Honey 3	2.860 ± 0.07	0.221 ± 0.01	1.733 ± 0.01	4.203 ± 0.10
Honey 4	3.266 ± 0.03	0.258 ± 0.01	1.850 ± 0.01	6.193 ± 0.006
Honey 5	2.716 ± 0.03	0.251 ± 0.006	1.383 ± 0.01	3.313 ± 0.10
Honey 6	1.893 ± 0.03	0.202 ± 0.002	1.513 ± 0.008	2.200 ± 0.10
Honey 7	2.263 ± 0.31	0.213 ± 0.01	1.610 ± 0.005	15.353 ± 0.26^{cd}

cd: $p < 0.0001$, d: $p < 0.001$

According to the Turkish Food Codex Honey Regulation (Anonymous, 2020), the proline content should be a minimum of 300 mg kg^{-1} (*Table 6*). The Turkish Food Codex Honey Regulation provides certain parameters, as shown in *Table 6*.

Table 6. *Characteristics of honey according to the Turkish Food Codex Honey Regulation*

Feature (100 grams of honey)	Flower honey	Secretory honey
Water **content** (maximum)	20%	20%
Sucrose **content** (maximum)	5-10 gr	5-10 gr
Fructose + **glucose content** (minimum)	60 gr	45 gr
Fructose/**glucose rat**io (minimum)	0.9-1.4 (thyme 1.0-1.65)	1.0-1.4
HMF (maximum)	40 mg/kg	40 mg/kg
Proline (minimum)	300 mg/kg	300 mg/kg
Diastase **number** (minimum)	8	8
Free **acidity** (maximum)	50 meq/kg	50 meq/kg

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Discussion

Coban et al. (2014) conducted a study on honeys produced in the Ardahan region, investigating their antioxidant capacities. The DPPH and ABTS free radical scavenging properties of honey samples were explored. According to the DPPH free radical scavenging results, it was determined that all honeys at a concentration of 200 µL exhibited antioxidant activity. The DPPH radical scavenging activity at 200 µL concentration ranged from 59.55% to 81.14%, at 400 µL concentration it ranged from 80.33% to 94.36%, and at 800 µL concentration, it ranged from 68.42% to 78.55%. Within this context, they identified the best DPPH radical scavenging activity at 400 µL concentration. When groups were compared based on concentration, it was determined that at 100 µL concentration, the values ranged from 0.86% to 21.29%, at 200 µL concentration it ranged from 0.613% to 39.96%, and at 400 µL concentration, it ranged from 13.31% to 79.44%, indicating a concentration-dependent radical scavenging effect. They observed that the ABTS radical scavenging effect increased in parallel with the concentration.

Bertonjelj et al. (2007) examined the antioxidant activities of seven types of honey samples from Slovenia. In their study, they found that light-colored linden and acacia honeys had significantly lower antioxidant activities compared to other honeys, while dark-colored spruce and wildflower honeys exhibited stronger antioxidant activities. Acacia honey had an antioxidant activity value of 0.07 mmol TE g^{-1}, while spruce and wildflower honeys were detected in the range of 0.42 to 0.47 mmol TE g^{-1} antioxidant activity values. Aljadi and Kamaruddin (2004) investigated the antioxidant properties of honey samples from two different sources in Malaysia. According to the research results, the DPPH value varied between 0.22 and 0.36 mg mL^{-1}. They determined that the total antioxidant activity ranged from 0.13 to 0.90 mmol TE g^{-1}. Kasirga (2019) determined the radical scavenging activity of honey samples produced in the Şırnak region using the DPPH test and found that the DPPH levels in honey samples ranged from 34.38 mg mL^{-1} to 229.22 mg mL^{-1}. They concluded that the color of honey was correlated with its antioxidant activity.

Our studies have led to the conclusion that, according to the data obtained and results from previous studies, the DPPH and ABTS radical scavenging activities generally increase with an increase in concentration.

The high fructose value in relation to the glucose value is consistent with findings in other scientific studies. Cavia et al. (2007) discovered in their studies that almost all forms of honey, except for a few with high glucose content such as canola honey, contain more fructose than other sugars. The high fructose content in these honeys indicates their quality. The similarity in fructose values among honeys in the region suggests that their qualities are similar.

Ozler et al. (2019) found, in their research on 17 honey samples collected from Konya and Karaman regions, fructose content ranging from %35.51 to %40.19, glucose content ranging from %26.47 to %33.70, and fructose/glucose ratio ranging from %1.10 to %1.41.

In a study conducted by Bengü and Kutlu in Bingöl and its surroundings, the sugar analysis ratio in the obtained honey was determined to be between 0.94 and 1.79 (Ozler et al. 2019). Another study on honeys from the Corum region investigated various quality parameters, including sugar content, in 47 honey samples. According to the results, the glucose ratio in honey samples was found to be 30.4%, fructose ratio was 35.3%, and sucrose ratio was 0.34% (Bahceci et al., 2015).

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A study focused on 30 samples of filtered flower honey produced in Iğdır and its surroundings examined the sugar values. The fructose value was determined as 38.62%, glucose value as 32.46%, fructose/glucose ratio as 1.20, and the total amount of glucose + fructose as 71.09%. According to these results, the filtered flower honeys produced in the Iğdır region generally comply with the TFC Honey Regulation (Yurt and Cakir, 2020).

The sugar composition of honey is one of the parameters that provides clues about possible adulteration in honey. Therefore, the amounts of sugars present in honey are crucial. The Turkish Food Codex Honey Regulation specifies the maximum allowable value for sucrose analysis as 5%, and all values obtained in our study comply with this regulation. The amount of sucrose in honey varies depending on the ripeness of the honey and the composition of the nectar. Early-harvested unripe honeys contain a higher amount of sucrose. If the amount of sucrose exceeds the specified limit in the honey standard, it may indicate adulteration (Tutkun, 2000).

Another quality criterion for honey is the HMF (5-hydroxymethylfurfural) value. Determining the amount of HMF in honey is used as an indicator of whether honey has been subjected to heat treatment and whether it is fresh or not. A low HMF value in honey indicates that the honey is fresh. Prolonged storage of honey or heat treatment can lead to the formation of dark-colored HMF due to fructose breakdown. However, it cannot be said that all dark-colored honeys have been stored for a long time or heated. If heat treatment is applied to dissolve crystallized honey, an increase in HMF value may be observed (Bahceci et al., 2015).

In the study conducted by Bengü and Kutlu (2018), the HMF values of honeys produced in Bingöl and its surroundings were determined to be in the range of 31.56% to 41.19%. In a study examining 47 honey samples collected from the Çorum region and investigating their compliance with the Turkish Food Codex Honey Regulation, the HMF values of honey samples were found to be 3.5 mg kg^{-1} (0.3-36.5) (20). Yurt and Çakır (2020) examined some properties of 30 samples of strained flower honey collected from different regions of Iğdır. The average HMF value of honey samples was found to be 39.85 ± 30.41 mg kg^{-1}. According to these results, some of the strained flower honeys produced in the Iğdır region were found to be compliant with the TFC Honey Regulation (Yurt and Cakir, 2020).

Honey, despite containing numerous amino acids, has a low protein content. Proline constitutes 50-85% of the total amino acid content. The amount of proline in honey varies depending on the plant it originates from. Although proline in honey is considered to come from natural pollen and nectar, the honeybee itself is indicated as the primary source. This is because the honeybee adds proline during the conversion of nectar into honey. Consequently, the amount of proline in honey is linked to the purity, maturity, and quality of the honey. Proline level is considered one of the most important factors in identifying counterfeit honey (Hermosin et al., 2003).

In the study conducted by Ozler et al. (2019), the proline content was found to be in the range of 349-908 mg kg^{-1}. In a study by Karamehmet (2021), the proline content of honey samples was found to be between 375.29 mg kg^{-1} and 532.68 mg kg^{-1}. In a study on Bangladeshi honey, Islam et al. (2012) mentioned that the proline amino acid level is an indicator of the antioxidant capacity of honey.

According to the TFC Honey Regulation, the minimum proline content in honey is 300 mg kg^{-1} and the results obtained are below this regulation. The proline values obtained in our study, when examined, range between 202 and 258 mg kg^{-1} after unit

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conversion, and it has been determined that the levels are below the threshold specified in the Honey Regulation. Many studies have reported proline values that meet the requirements of the Honey Regulation; however, there are places in both Turkey and other countries where proline values are below 300 mg kg^{-1}. Upon analysis of the data obtained in our study, it is observed that the levels fall below the regulation.

It is known that proline content is low or absent in honey exposed to excessive heat, stored for a long time, or in adulterated honey (Can et al., 2015; Duymaz, 2020). Therefore, the proline content of honey is an important criterion in evaluating its quality and purity, and according to the Turkish Food Codex Honey Regulation, the proline amino acid content of honey should be at least 300 mg kg^{-1} (Anonymous, 2020).

In our study, biochemical analyses were conducted to determine the total phenolic content, which is a natural antioxidant in the honey samples we used. The amount of total phenolic compounds in honey varies depending on the geographical structure of the place where the honey is produced and the methods used to reduce sugar interactions. There are studies in the literature comparing the phenolic content of honey obtained from various sources.

Sagdic et al. (2013) stated in their study that the phenolic content of honeys produced in Turkey, with different botanical sources, ranged from 15 to 1082 mg GAE kg^{-1}. In another study, it was mentioned that the phenolic content of Turkish honeys ranged from 138 to 1048 mg GAE kg^{-1} (Bahceci and Guzel, 2020). Gul and Pehlivan (2018), in their analysis of 23 different honey samples collected from various regions of Turkey, found the total phenolic content to be in the range of 34.37 to 470.70 GAE 100 g^{-1}.

In our research, the total phenolic content of 7 honey samples from Hizan district of Bitlis province was determined to be in the range of 1.243 to 2.203 mg g^{-1}. It was concluded that the total phenolic content of these honey samples showed parallel values compared to other honey samples in our country.

In our study, the analyses of flavonoid values of the honeys from the Hizan region were conducted. The obtained values were compared with the flavonoid values obtained in previous studies. Flavonoids can contribute some antioxidant properties to honey besides color. It has been observed in previous studies that flower honeys generally contain more flavonoids quantitatively compared to honeydew honeys, and there are variations in flavonoids among different types of honey (Karadal and Yildirim, 2012). The antioxidant properties of flavonoids are their most significant biological characteristics. Many researchers are interested in flavonoids because they function as free radical scavengers, enzyme regulators, antibiotics, anti-allergens, antidiabetics, anti-ulcer, and anti-inflammatory agents (Cakici and Yassihuyuk, 2013; Silva et al., 2021).

Conclusion

As a result, we have found variations in the antioxidant capacity, HMF content, sugar content, proline, and other parameters of honey samples from the Hizan region used in our study. The composition of honey can differ in terms of the number and variety of phytochemical compounds present. This is mainly due to factors such as sunlight, seasonal changes, altitude, bee species, the plant flora from which honey is harvested, and the honey harvesting period.

It has been determined that the honey samples used in our research are as close to natural honey as possible, free from imitation and adulteration, and that significant

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intervention was not made during production. As a result of the diversity of plants in the Hizan region, it was concluded that the biochemical contents of honey, except for the proline value, comply with the Turkish Food Codex honey regulation. Consequently, after investigating the reason for the low proline value in the evaluated honey samples and raising it to the desired level, it can be said that they can be introduced to the domestic market and are of high quality for consumers.

When the examined honeys are evaluated based on their naturalness, honeys numbered 1 and 4, which have the highest phenolic content, are considered more natural compared to other honeys. Additionally, the presence of a high level of flavonoids in honeys numbered 1 and 7 suggests that these honeys could be used medically and require further investigation.

The maltose level within the specified range is thought to be a result of using very little or no honeycomb during the production process. The carcinogenic, cytotoxic, and mutagenic effects of HMF and its derivatives, discovered in recent scientific research, emphasize the importance of these values for consumer health. The freshness of honeys and whether they have been stored for a long time or exposed to high temperatures can be understood by looking at the measurement results of HMF activity. The HMF values clearly indicate that the honey samples used in our study were stored under appropriate conditions and did not undergo any heat treatment.

In conclusion, as the specified HMF and its derivatives comply with standards, the honey samples produced in the Hizan region do not pose any risk to public health. Considering all these results, it can be inferred that these honey samples, produced in the Hizan region, generally meet the standards set by the Turkish Food Codex, are produced under natural conditions, and can be safely consumed. Based on these findings, encouraging more efficient honey production by beekeepers in Hizan is believed to benefit the local and national economies. Beekeepers should be educated in this regard, taking into account scientific studies and the results of such research to facilitate the realization of quality/natural honey production. The Ministry of Agriculture should conduct necessary inspections and provide support through incentives. Warning should be issued and penalties imposed on businesses engaged in the production of counterfeit honey that poses a threat to human health, and measures should be taken to prevent such production.

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