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QUALITY EVALUATION OF PINE AND BLOSSOM HONEY SAMPLES PRODUCED IN TURKEY: CORRELATION BETWEEN PHYSICOCHEMICAL CHARACTERISTICS

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ABSTRACT
This study presents physicochemical characteristics of 39 honey samples (21 blossom and 18 pine honey) collected during two years from three different geographical regions of Türkiye that differs vastly in climatic conditions and thus plant species. The samples were analysed for $\delta^{13}C/\delta^{12}C$ stable carbon isotope ratios of honey ($\delta^{13}C_h$) and its protein fraction ($\delta^{13}C_p$), moisture, free acidity, proline and 5-hydroxymethyl furfural (HMF) content, diastase activity and sugar composition. The results showed that C_4 sugar content, proline content, diastase activity, acidity values of pine honeys were higher than that of blossom honeys whereas, higher moisture and HMF content were detected for blossom honeys. Besides, geographical region mainly affected the moisture and C_4 sugar contents. High correlations between HMF and $\delta^{13}C_h$ and $\delta^{13}C_p$; proline and acidity values; fructose and glucose content were determined, and this indicated the robustness of the analysis and quality evaluation among different honey types and regions.
Keywords: Honey, physicochemical characterization, geographical variability, carbon isotope ratio, hydroxymethyl furfural

TÜRKİYE'YE ÖZGÜ ÇAM VE ÇİÇEK BALLARININ KALİTE ÖZELLİKLERİNİN DEĞERLENDİRİLMESİ: FİZİKOKİMYASAL ÖZELLİKLER ARASINDAKİ İLİŞKİ

ÖZ
Bu çalışma, Türkiye'nin iklim koşulları ve dolayısıyla bitki türleri bakımından büyük farklılıklar gösteren üç farklı coğrafi bölgesinden iki yıl boyunca toplanan 39 bal örneğinin (21 çiçek ve 18 çam balı) fizikokimyasal özelliklerini ortaya koymaktadır. Örnekler, balın $\delta^{13}C/\delta^{12}C$ kararlı karbon izotop oranları ($\delta^{13}C_h$) ve bunun protein fraksiyonu ($\delta^{13}C_p$), nem, serbest asitlik, prolin ve 5-hidroksimetil furfural (HMF) içeriği, diastaz aktivitesi ve şeker bileşimi açısından analiz edilmiştir. Sonuçlar, çam ballarının C_4 şeker içeriği, prolin içeriği, diastaz aktivitesi ve asitlik değerlerinin çiçek ballarından daha yüksek olduğunu, çiçek ballarında ise nem ve HMF içeriğinin daha yüksek olduğunu göstermiştir. Ayrıca coğrafi bölge faktörü, nem ve C_4 şeker içeriklerini büyük ölçüde etkilemiştir. HMF içeriği ile

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$\delta^{13}C_h$ ve $\delta^{13}C$ değerleri arasında, prolin ve asitlik değerleri arasında ve fruktoz ve glukoz içerikleri arasında yüksek korelasyonlar tespit edilmiş ve bu durum farklı bal türleri ve farklı bölgeler arasında yapılan analiz ve kalite değerlendirmelerinin geçerliliğini göstermiştir.
Anahtar kelimeler: Bal, fizikokimyasal karakterizasyon, coğrafi farklılıklar, karbon izotop oranı, hidroksimetil furfural

INTRODUCTION

Honey is a natural product that honeybees (*Apis mellifera*) produce by collecting and processing plant nectars (Mohammed and Babiker, 2009). It is known as a valuable substance that has high nutritional properties and therefore can be used in many medicinal practices (Hernández et al., 2005). Several factors can affect honey quality such as plant variety that bees gather their nectar needs, climatic factors of the plant area, process and storage conditions. For this reason, investigating honey quality regarding the essential properties is very important considering the health of consumer (Abdulkhaliq and Swaileh, 2017).

In Türkiye, honey production is well developed and beekeeping is an important agricultural activity that has been going on for many years due to the environmental conditions that ensure a proper atmosphere for apicultural activities geographically and climatically (Kahraman et al., 2010). Türkiye is recognized as one of the biggest honey producers worldwide (Yardibi and Gumus, 2010). According to the FAO statistics (FAO, 2022), there are about more than 8 million hives in Türkiye and c.a.111.000 tons of honey have been produced by 2022 totally.

Blossom honey is produced from nectar collected directly from flowers by worker bees, and is the most popular honey type consumed and commercially available in Türkiye. In general, this honey is produced from a variety of flowers and thus called as a "poly-floral" honey that doesn't have any particularly distinct flavour. Unlike blossom honey, pine honey is actually produced from honeydew, which is a liquid secreted from a type of insect that lives on pine trees. Türkiye is the global leader in the production of pine honey, with more than 90% of the total output originating from Türkiye (Sarı, 2022).

More than two hundred components are defined in honey (Escuredo et al., 2013) and the main ones are sugars (70–80%) and water (10–20%). The remaining part is composed of free amino acids, proteins, minerals, vitamins, phenolic acids and organic acids. Honey properties and composition differ widely according to the region, season, bee species, plants variety in the region, harvesting method, and storage period in honeycomb and postharvest storage. Considering that there are many floral origin possibility, it is expected that a honey is not exactly the same as the other (Kirs et al., 2011).

The honey quality is identified relaying on its unique characteristics such as physical, chemical, sensorial and microbiological properties (Finola et al., 2007). The regulations and standards regarding honey are developed to ensure standardization of the processing and clearness in the market. The necessary conditions to assess the honey for its authenticity are stated by the Codex Alimentarius, whose main purpose is to assign the significant quality limits that are important for consumption.

Honey adulteration is one of the main issues that need attention for honey manufacturers and also for the consumers. Not only it affects honey producers economically but also it has unfavourable impacts for consumer health. Honey fraud via bee feeding by a variety of sugars during honey production or manipulating honey after production by adding different sugar syrups are some of the ways for adulteration that result in change in the composition of pure honey. Starch-based sugar syrups, high fructose corn syrup, glucose syrup and sucrose syrups are some of the sugars which can be used (Tosun, 2013). Standard $\delta^{13}C/\delta^{12}C$ carbon isotope ratio analysis is well recognized for the detection of fraud in honey with sugar cane or corn (C_4) sugar syrups (Elflein et al., 2008; Simsek et al., 2012; White and Winters, 1989).

Currently honey market tends to define geographical limits for production with the purpose of providing a particular standard of quality developed and marketed for a production area. One region could produce better quality honey products than others. Thus, labelling of regional honey must be supported by analysis in order to confirm its origin (Viuda-Martos et al., 2010). Numerous studies reported the physicochemical characteristics of several types of honey from various geographical regions around the world (Al-Khalifa and Al-Arify, 1999; Kahraman et al., 2010; Mendes et al., 1998; Ouchemoukh et al., 2007). However, there are limited number of studies exhibiting the chemical composition and quality parameters of Turkish commercial honeys. In particular, there is limited study in which the quality characteristics of commercial pine and blossom honey obtained from different regions of Türkiye are determined comprehensively and associated with their geographical regions. The purpose of the present work is to exhibit the physicochemical characteristics of blossom and pine honey samples collected in two years from three different regions of Türkiye, namely, Black Sea, Central Anatolia, and Mediterranean Regions where the climatic conditions and plants varies significantly. Besides, the correlation among the quality parameters has been assessed as well as they are evaluated with regard to national and international honey standards.

MATERIAL AND METHODS

Honey samples

Commercial honey samples (21 blossom and 18 pine) were obtained from commercial producers in Black Sea (6 blossom and 5 pine honeys), Central Anatolia (5 blossom and 4 pine honeys) and Mediterranean Region (10 blossom and 9 pine honeys) in the year of 2013 and 2014. The regions where honey was collected are shown in Fig. 1. The samples were stored in a refrigerator until taken to analysis.

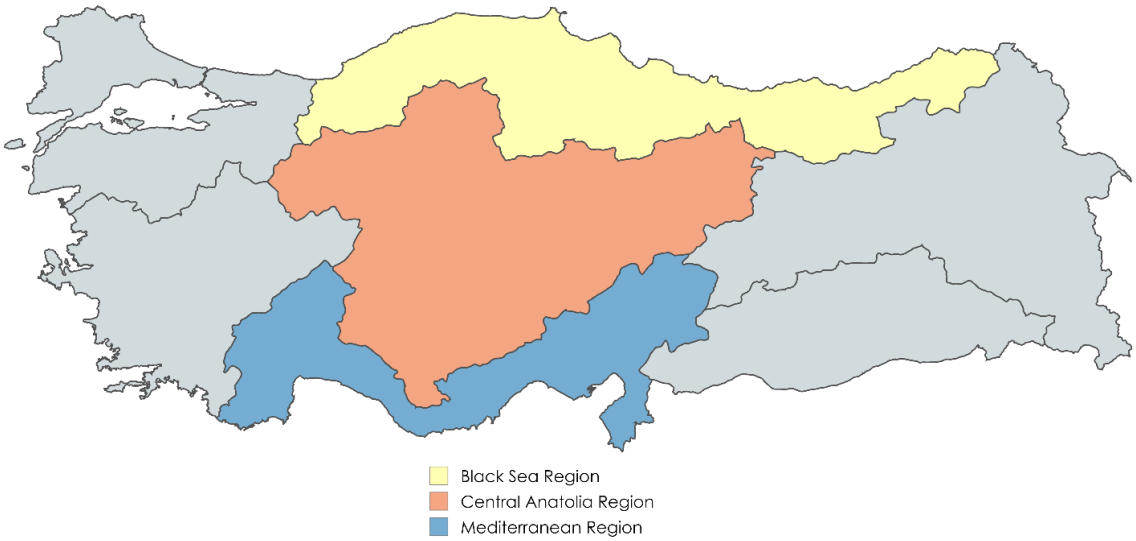

Figure 1. The regions where honey was collected in Türkiye

Moisture content

Moisture content was measured by a digital refractometer (Atago, Japan) according to AOAC 969.38 and 920.180 methods.

Acidity

Total acidity was measured with regard to the harmonized method as described by Bogdanov (2009). Briefly, 10 g of the sample were dissolved in 75 ml CO_2-free distilled water and titrated with 0.1 M NaOH until the pH reached 8.5.

Stable carbon isotope ratio

$\delta^{13}C_h$ and $\delta^{13}C_p$ was analysed by an isotope ratio mass spectrometry system coupled with an elemental analyser (EA-IRMS, Thermo Scientific, Germany) based on AOAC 998.12 method. As reference materials to draw linear calibration curve sucrose (RM-8542 from NIST, USA) and L-glutamic acid (RM8573 NIST, USA; USGS40) were used whose $\delta^{13}C$ (delta) values are -10.47±0.13‰ and -26.24±0.07‰, respectively. Graphite (RM8541, NIST, USA) was used in order to control accuracy. CO_2 with $\delta^{13}C$ (delta) values −41.04‰ was utilized as reference gas. Amount of C_4 sugar was calculated according to following equation (Eq.1) provided by Padovan et al., (2007), where $\delta^{13}C_s$ is accepted as -9.7 for corn sugar.

$$C_4\ (\%) = [(\delta^{13}C_p - \delta^{13}C_h) / (\delta^{13}C_p - \delta^{13}C_s)] \times 100 \quad \text{(Eq.1)}$$

5-hydroxymethyl furfural content

HMF was determined by dilution of honey with distilled water and addition of p-toludine solution according to the method of IHC, Determination of HMF After Winkler (Bogdanov, 2009). Absorbance was determined at 550 nm using a 1 cm cell in a double beam spectrometer (Perkin Elmer, England). The content of HMF was calculated as follows:

$$\text{HMF} = \frac{192 \times A \times 10}{\text{Weight of honey in grams}} \quad \text{(Eq.2)}$$

Where A is the absorbance and 192 is the factor for dilution and extinction coefficient. Results were expressed as mg/kg.

Diastase activity

The diastase activity was measured based on the method of AOAC 958.09 by using a buffer solution of soluble starch and honey which was incubated in a special glass test tube. Results were expressed as ml of 1% of starch hydrolysed by an enzyme in 1 g honey in 1 hour.

Sugar composition

The fructose, glucose and sucrose contents were determined by HPLC (Shimadzu 10-A, Japan), using a refractive index (RTP-6A) detector, on 250 x 4.6 mm x 5 micron NH_2 column. Sample preparation and chromatographic procedure were conducted as described in DIN 10758. Briefly 1.3 ml/min of pump flow, 30±1°C of column temperature, 10 µl of injection volume and acetonitrile:water (80:20, v/v) mixture for mobile phase were used in the study.

Proline content

Proline content was determined by AOAC 979.20 method by the measurement of the absorbance at 510 nm of the resulting product between proline and ninhydrin in an acidic medium.

Statistical Analysis

Statistical analysis was conducted by SPSS version 20.0 for Windows (IBM corp, USA). The statistical differences between the physicochemical properties of the samples were evaluated according to honey type and geographical region through two-way analysis of variance (ANOVA) followed by LSD post hoc test. Differences between mean values at the 95% ($p < 0.05$) confidence level was considered statistically significant. Correlations were obtained by Pearson's correlation coefficient (r) in bivariate linear correlations. The strength of the correlation were described using the guide that Wuensch and Evans (1996) suggested for the absolute value of r, namely, "Moderate correlation" was defined as $0.40 \leq |r| \leq 0.59$; "strong correlation" was defined as; $0.60 \leq |r| \leq 0.79$ and "very strong correlation" was defined as; $0.80 \leq |r| \leq 1.00$.

RESULTS AND DISCUSSION

The mean values and standard deviations of various physicochemical parameters, as well as national limits and number of samples exceeding relative limits are given in Table 1 for blossom and pine honeys. In order to outline the general quality characteristics of the commercial honey samples in the presented study, their compliance with national and international standards was examined. Overall, 97.4% of samples were fully in the range of the quality parameter limits set on Turkish Food Codex (2020) and European Commission Regulation (European Commission, 2002). Out of 39 samples, only one sample which is a pine honey from Mediterranean Region was out of the limit values due to its sucrose content.

Table 1. The quality properties of blossom and pine honeys from three regions of Turkey

Region	Northern Anatolia		Middle Anatolia		Southern Anatolia		Limits in Turkish Food Codex
Honey (N)	Blossom (6)	Pine (5)	Blossom (5)	Pine (4)	Blossom (10)	Pine (9)	
$\delta^{13}C_h$ ‰	-24.45a (0.56)	-23.67b (0.55)	-24.36a (0.61)	-24.35b (0.86)	-24.78a (0.59)	-24.26b (0.35)	≤ -23* ≤ -22.5**
$\delta^{13}C_p$ ‰	-25.19a (0.70)	-24.70a (0.57)	-24.92a (0.56)	-24.83a (0.45)	-25.09a (0.58)	-24.86a (0.56)	N.A
$\delta^{13}C_p$-$\delta^{13}C_h$ ‰	-0.73a (0.30)	-1.03b (0.21)	-0.56a (0.24)	-0.48b (0.45)	-0.31a (0.27)	-0.60b (0.32)	≥ -1* N.A**
C_4 %	4.70a (1.89)	6.19b (0.56)	3.66a (1.59)	3.25b (3.09)	2.02a (1.72)	3.91b (2.04)	≤ 7* N.A**
Acidity (meq/kg)	23.27a (3.70)	24.41a (4.37)	21.9 a (4.21)	25.35a (4.04)	24.64a (2.78)	24.86a (4.13)	≤50
Moisture %	17.77a (0.88)	16.68c (0.33)	16.23a (0.15)	15.64b (0.90)	17.29a (0.40)	16.54b (0.53)	≤20
Diastase activity	10.02a (1.41)	12.73b (2.29)	10.49a (1.88)	10.77b (2.10)	11.62a (2.98)	14.24b (3.43)	≥8
HMF (mg/kg)	20.19a (10.88)	10.18a (9.94)	22.80a (14.57)	16.20a (6.47)	24.28a (10.99)	14.40a (7.42)	≤40
Proline (mg/kg)	492.84a (55.59)	505.61a (139.24)	463.84a (67.45)	529.95a (50.42)	503.94a (38.96)	546.45a (78.69)	≥300
Fructose %	38.25a (0.69)	33.77b (0.61)	36.61a (2.12)	32.87b (1.74)	35.68a (2.64)	33.16b (4.79)	N.A
Glucose %	34.01a (0.69)	28.09b (1.31)	29.53a (1.47)	27.99b (0.56)	30.93a (2.69)	28.05b (3.54)	N.A
Sucrose %	0.55a (0.19)	0.67a (0.56)	0.78a (0.44)	0.76a (0.74)	0.38a (0.34)	0.41a (0.45)	≤5
F+G %	72.26a (0.87)	61.87a (1.74)	66.14a (3.28)	60.86a (1.73)	66.61a (5.18)	61.21a (8.22)	≥60* ≥45**
F/G %	1.13a (0.03)	1.20b (0.05)	1.24a (0.06)	1.18b (0.07)	1.16a (0.05)	1.18b (0.06)	(0.9-1.4)* (1.0-1.4)**

Samples for each region and honey types are analyzed with two replicates; mean values are presented and standard deviations are given in parenthesis; a-b different letters in the same line show statistically significant differences ($P < 0.05$); different colors (□, □, □, □) in the same line shows the statistically significant differences between the geographic regions in the ($P < 0.05$); F: fructose; G: glucose, N: Sample number; *: blossom honey, **: pine honey; N.A: not available

Carbon isotope ratio ($\delta^{13}C/\delta^{12}C$) and C4 sugar amount in honey samples

Honey adulteration is one of the most challenging food quality issues in the world in terms of difficulty in detection. Adulteration is generally implemented by mixing honey with glucose and/or fructose from cheaper resources. Therefore, advanced analytical techniques are needed in order to differentiate the added sugar to honey due to its essential composition is based on glucose and fructose, already. Recently developed carbon isotope ratio analysis, enables to monitor this type of adulteration using EA-IRMS system (Tosun, 2013). In this method, $\delta^{13}C/\delta^{12}C$ values of both honey ($\delta^{13}C_{h)}$ and protein fraction of honey ($\delta^{13}C_p$) were analysed. The difference between them was used as an indication of adulteration both qualitatively and quantitatively (Padovan et al., 2003). When sugar from C_4 plants is added to pure honey, the $\delta^{13}C_h$ value will be altered, whereas its corresponding $\delta^{13}C_p$ value will remain constant. This is based on

the differences between the CO_2 fixating pathways of plants in photosynthesis where they are divided into three groups, accordingly: C_3, C_4 and CAM. C_3 plants (such as sugar beet, apple, grapes, etc.) fixate CO_2 into 3-carbon compound, whereas C_4 plants (such as corn and sugar cane) fixate into 4-carbon compound. Honeybees are generally use C_3 plants, and this fact affects directly the $\delta^{13}C/\delta^{12}C$ ratio values of the honey. This ratio was measured as -21.9‰ to -30.4‰ and -11.8‰ to -19.0‰ for honeys generated from C_3 and C_4 plants, respectively (Martin et al., 1998). That difference is used to detect the fraud of external sugar addition to honey from C_4 plants. However, this criterion is lacking when the honeybees use C_3 plants together with C_4 ones, or if the C_4 sugar addition is in low amount to change the isotope ratio. Therefore, $\delta^{13}C/\delta^{12}C$ ratio of raw honey is evaluated together with the $\delta^{13}C/\delta^{12}C$ ratio value of honey's protein fraction, in order to make a concrete evaluation of honey adulteration due to its constant value, even after C_4 sugar addition to honey (Çınar et al., 2014). The difference between the $\delta^{13}C/\delta^{12}C$ values of honey and its protein fractions is denoted as $\delta^{13}C_p$-$\delta^{13}C_h$ in Table 1. Its higher values than 1‰, is corresponding to C_4 sugar addition and considered as honey adulteration (Padovan et al., 2007; Simsek et al., 2012; Tosun, 2013; White et al., 1998).

In the presented study, the $\delta^{13}C/\delta^{12}C$ values of the raw honey and its protein fraction counterparts for blossom honeys were detected in the range of -25.62‰ to -23.52 ‰ and -26.08‰ to -23.93‰, respectively whereas the pine honey samples are indicated a carbon isotope ratio from -25.04‰ to -22.90 ‰ and protein extracts from -25.94‰ to -23.95 ‰ (data not shown in Table 1). The mean values were calculated with regard to honey types and their originated region (Table 1). All those values were in accordance with the ones reported for Turkish blossom and pine honeys in literature and also with the national and international limits (Tosun, 2013). Moreover, there were statistical differences between carbon isotope ratio values with regard to type and region of the honeys. $\delta^{13}C_h$ values of blossom honeys were slightly higher than that of pine honey samples. This may be attributed to the higher fructose and glucose composition of blossom honey samples, which may induce higher $\delta^{13}C_h$. Pine honey is a unique type of honeydew honey and 90% of world's pine honey is produced in Türkiye. Whereas, there was no significant difference between $\delta^{13}C_p$ values, which indicates the constant value of protein fraction. Interestingly, there were also significant differences among the $\delta^{13}C_p$-$\delta^{13}C_h$ and C_4 values between pine and blossom honeys as well as their originated region. The highest values detected in honey samples from Northern regions among others (Table 1). This can be explained by the dependency of honey quality to extrinsic factors such as climatic factors and originated plant which varies significantly among geographical regions of Türkiye. Those results were also compatible with correlation results presented in Table 2. There was a very strong correlation between $\delta^{13}C_p$-$\delta^{13}C_h$ and C_4 values with a correlation coefficient of 0.983 (Table 2). This indicates the consistency of interpretation of $\delta^{13}C_p$-$\delta^{13}C_h$ values as honey adulteration via C_4 sugar addition. Besides, the strong correlation between $\delta^{13}C_p$ and $\delta^{13}C_h$ parameters with a high coefficient value is an indirect indicator of robustness of applied method.

Acidity

Free acidity is an important property related to the deterioration of honey and it shows variability among honey types. It may be explained as an indicative parameter for fermentation of sugars into organic acids especially the gluconic acid (Al-Khalifa and Al-Arify, 1999; Kirs et al., 2011). The free acidity values of the blossom honeys in this study ranged between 17.79 and 29.93 meq/kg and of pine honeys between 17.86 and 33.96 meq/kg (data not shown in Table 1). All samples were below the limit permitted by national and international authorities (50 meq/kg), demonstrating the freshness of Turkish commercial honeys and absence of undesired fermentations. Besides, no significant difference was observed among the acidity values of blossom and pine honeys from different regions.

Table 2. Pearson correlation coefficients of the physicochemical properties.

	$\delta^{13}C_h$	$\delta^{13}C_p$	$\delta^{13}C_p$ -$\delta^{13}C_h$	C_4 sugars	Proline	Diastase	HMF	Acidity	Moisture	Fructose	Glucose	Sucrose	F+G	F/G
$\delta^{13}C_h$	1													
$\delta^{13}C_p$	**0.745***	1												
$\delta^{13}C_p$-$\delta^{13}C_h$	-0.424	0.238	1											
C_4sugars	**-0.507***	0.156	**0.983***	1										
Proline	0.252	0.431	0.161	0.125	1									
Diastase	-0.083	-0.071	0.018	0.071	0.390	1								
HMF	**0.557***	**0.572***	0.049	-0.030	0.247	-0.283	1							
Acidity	0.174	0.273	0.079	0.049	**0.567***	-0.067	0.349	1						
Moisture	0.416	0.397	-0.074	-0.089	0.140	-0.018	0.261	0.196	1					
Fructose	0.050	-0.004	-0.046	-0.096	-0.332	-0.427	0.073	-0.243	0.143	1				
Glucose	0.173	0.023	-0.151	-0.210	-0.190	-0.338	0.153	-0.157	0.408	**0.874***	1			
Sucrose	-0.173	-0.415	-0.264	-0.246	-0.261	-0.085	-0.201	0.071	-0.144	0.029	0.030	1		
F+G	0.113	0.010	-0.100	-0.156	-0.272	-0.397	0.115	-0.208	0.280	**0.970***	**0.966***	0.030	1	
F/G	-0.230	-0.024	0.235	0.253	-0.253	-0.165	-0.138	-0.125	-0.522	0.152	-0.344	-0.029	-0.090	1

"Strong" ($0.60 \leq |r| \leq 0.79$) and "very strong" ($0.80 \leq |r| \leq 1.00$) correlations are in bold and significant * P < 0.01.

In a previous study, Gürbüz et al., (2020) reported a wider range of free acidity values for blossom honey samples collected from Southeastern Türkiye, ranging from 2.00 to 44.00 meq/kg. On the other hand, Güzel and Bahçeci (2020) determined the free acidity of blossom honeys from Northern Türkiye to be between 21.10 and 47.80 meq/kg. In case of pine honeys, Uçurum et al., (2023) conducted analyses on pine honey samples from the western part of Türkiye, finding free acidity levels ranging from 8.00 to 46.89 meq/kg, with an average value of 18.57±5.62 meq/kg. In a study conducted in Romania, pine honeys and polyfloral honeys exhibited free acidity levels within the range of 11.80-20.00 meq/kg and 5.20-37.10 meq/kg, respectively, signifying acceptable quality and a low degree of deterioration (Oroian et al, 2017). All these differences among regions may be attributed to variations in the flora from which bees collect nectar, as well as the sample sizes employed in each study.

Moisture content

Moisture content as a noteworthy property affects the physical quality of honey such as viscosity, crystallization, appearance, aroma, specific gravity, solubility and preservation (Escuredo et al., 2013). The maximum amount of water contained by honey is important due to the risk of fermentation and granulation during storage. Moisture content in honeys analysed in this study ranged from 14.50% to 18.90% for blossom honeys and from 15.65% to 17.20% for pine honeys (data not shown in Table 1). All samples in the study included less than 20% water, which is the maximum limit defined by national and international regulations. In a study conducted by Güzel and Bahçeci (2020), the moisture content of blossom honeys from Northern Türkiye was determined as to be between 14.5% and 21.7%. On the other hand, Gürbüz et al., (2020) reported a range from 14.04% to 16.68% for blossom honey samples belonging Southeastern Türkiye. In another study, the moisture content ranged from 15.40% to 18.80% for blossom honeys in Central Anatolia (Özler et al., 2019). In research carried out in Romania, it was found that pine honeys had moisture levels ranging from 14.44% to 17.20%, while polyfloral honeys showed moisture content within the range of 15.43% to 19.64%, which is also similar to present study (Oroian et al., 2017). Honey's water content varies according to several factors including the relative humidity of the region or the season (Karabagias et al., 2014). This may explain the significant difference in moisture contents of honeys from different regions (Table 1). The moisture contents of honeys from humid climate (Northern and Mediterranean Regions) were higher than arid climatic regions such as Central Anatolia of Türkiye. Moreover, due to the property of honey

as being hygroscopic and absorbing moisture from the environment, the moisture in honey can also rise depending on the process parameters and unsuitable storage conditions. Those could explain the significant difference in moisture contents of flower and pine honeys from different regions (Table 1). On the other hand, the water content in honey plays a key role in its resistance to fermentation. Honey with a higher moisture level is more prone to fermentation over time due to the growth of sugar tolerant yeasts (Singh and Singh, 2018).

Diastase activity and HMF

Diastases are classified as amylolytic enzymes that contain α- and β-amylases and constitute a small portion of the proteins present in honey naturally. Diastase activity is affiliated with many factors such as the geographical and floral origins of the honey (Ahmed et al., 2013). Besides, diastase content in honey may differ according to the age of the honeybees, the nectar collection and the colony's physiological season, the amount of nectar and its sugar composition because a high quantity of nectar results in a lower enzyme content and lower pollen consumption (P. M. Da Silva et al., 2016). Moreover, diastases are thermo-labile and their amount decrease if the honey is subjected to heating or if it is stored for a long time. Consequently, diastase content is used for the evaluation of honey freshness and/or overheating (above 60^{o}C) of the product (Ahmed et al., 2013). Therefore, diastase activity needs to be evaluated along with HMF content for detecting the freshness and/or overheating. International authorities determine the minimum value of diastase activity as 8 on Göthe's scale and maximum limit of HMF as 40 mg/kg (European Commission, 2002). Diastase activity in this study was found in the range of 8.07-17.79 for flower honeys and 8.31-20.35 for pine honeys (data not shown in Table 1). The mean values differed significantly among honey types (Table 1), pine honeys contained slightly higher amount of diastase activity than flower honeys and similar results were reported in literature (Ünal and Küplülü, 2006; Vorlová and Čelechovská, 2002). As it is seen in Table 2, a negative correlation (r = -0.283) was observed between diastase activity and HMF. This result also indicated the negative correlation between the decrease in the concentration of diastase due to its sensitivity to heat treatment and the formation of HMF as a result of exposure to heat treatment. There was also moderate correlation (r = -0.427) between diastase activity and fructose content. Thrasyvoulou (Thrasyvoulou, 1986) found the loss of diastase activity is associated with fructose (r = -0.67) and glucose content (r = -0.48), which is in agreement with the present study.

The content of HMF in honeys ranged from 9.22 to 39.46 mg/kg and from 1.73 to 34.18 for flower honeys and pine honeys, respectively (data not shown in Table 1). The presented results demonstrated a high level of quality of all commercial honey samples in this paper in accordance with national and international regulations (Table 1). HMF as a by-product in Maillard reaction or a decomposition product of monosaccharides especially appears when honey is subjected to heating or storage for a long period. While the heat treatment intensity and the storage period of the honey increase, the HMF content also increases substantially. Tornuk et al., (2013) analysed twenty Turkish flower honeys by HPLC/DAD and the values ranged between 0 and 4.12 mg/ kg, which is lower than that of found in this paper. Nevertheless, HMF alone cannot be evaluated as a parameter to detect the severity of the heat processing, because other factors such as the sugar profile, presence of organic acids, pH, and moisture content can influence the levels of HMF. That's why; the HMF value can only be an indicative parameter for overheating or improper storage. In the presented study, there was no statistical difference between HMF values of pine and flower honeys and among geographical regions, which can be attributed to the high standard deviation values of the results (Table 1). According to correlation data in Table 2, HMF content was strongly correlated with $\delta^{13}C/\delta^{12}C$ ratios of honey (r = 0.557) and its protein fraction (r = 0.572). This high correlation may be due to HMF is formed from reducing sugars in honey in acidic environments when they are heated through the Maillard reaction.

Proline content

Amino acids comprises 1% (w/w) of the whole components of honey and proline is known as the most abundant amino acid in honey and pollen (P. M. Da Silva et al., 2016). Relative proportions of amino acids vary according to the origin of the honey (Hermosín et al., 2003). Proline corresponds a total of 50–85% of amino acid quantity in honey (Iglesias et al., 2006). It originates mainly from the salivary secretions of honey bees during the conversion of nectar into honey and it is used as a criterion for the evaluation of the maturation of honey, and in some cases, adulteration with sugar. Besides, the content of proline is associated with the antioxidant capacity of honey (Bentabol Manzanares et al., 2011). The minimum limit for authentic honey is determined as 300 mg/kg for proline value according to Turkish Food Codex (2020). In this study, the amount of proline in blossom honeys ranged from 389.29 to 594.64 mg/kg, while in pine honeys, it was found to be within the range of 357.83 to 638.27 mg/kg (data not shown in Table 1). Thus, in accordance with the criterion, all honeys analysed in this study were ripened and not adulterated (Table 1). In a study conducted by Özler et al., (2019), the proline content of polyfloral honeys were determined as to be between 349-908 mg/kg. On the other hand, Gürbüz et al., (2020) reported a range from 117.15-933.49 mg/kg for blossom honey samples belonging Southeastern Türkiye which indicates that proline amount of some samples was found less than 300 mg/kg, which was the legal minimum limit.

As it is expected there was positive correlation ($r = 0.431$) between $\delta^{13}C/\delta^{12}C$ ratios of honey protein fraction and proline content (Table 2). Interestingly, a positive moderate correlation ($r = 0.567$) was observed between acidity and proline content (Table 2) which may be due to the acidic characteristic of proline.

Sugar content

Sugar composition depends mainly on the honey type, geographical origin, and varies according to the climatic changes, process and storage conditions (Escuredo et al., 2013; Tornuk et al., 2013). Fructose, glucose and sucrose are the major components of honey (Kahraman et al., 2010). In this study, the predominant sugar in all analysed honey samples was fructose. As it is presented in Table 1, the mean values of fructose content were higher than the glucose content in all honey samples. Besides both fructose and glucose contents of flower honeys were significantly higher than that of pine honey samples. In many studies (Golob and Plestenjak, 1999; Mateo and Bosch-Reig, 1997) it was reported that lower glucose and fructose levels in honeydew honeys than in their blossom honey counterparts. The content of these invert sugars and the ratio between them, are important indicative parameters for honey classification. In almost all honeys, fructose is predominant sugar excluding some honeys like rape (*Brassica napus*) and dandelion (*Taraxacum officinale*). In these honey types glucose content could be higher than fructose content (Escuredo et al., 2013) which often results in rapid crystallization. The fructose/glucose ratios were widely distributed, indicating the variety of plant sources that bees used for honey production (Al-Khalifa and Al-Arify, 1999). The fructose/glucose ratio which affects honey flavour and may result in crystallization was calculated for all honey samples in this study and it ranged between 1.08 and 1.29 for flower honeys and 1.07 and 1.28 for pine honeys. Sum of fructose and glucose content varied from 58.76 to 76.88 for flower honeys and from 46.10 to 74.03 % for pine honeys (data not shown in Table 1). The fructose/glucose ratio and sum of fructose and glucose values reported in Table 1 as mean values were acceptable according to the regulations which set fructose/glucose ratio as 0.9-1.4 for flower honeys and 1.0-1.4 for pine honeys and sum of fructose and glucose as minimum 60 g/100 g for flower honeys and minimum 45 g/100 g for pine honeys. As it was expected, very strong correlations were detected between glucose content, fructose content and the sum values (Table 2).

The sucrose level varies depending on the maturity level and source of the nectar used for honey (Kahraman et al., 2010). The mean values of sucrose content were given in Table 1; the overall mean values were detected as 0.57 g/100g

for flower honeys and 0.61 g/100g for pine honeys. Out of 39 samples, only one sample which is a pine honey from Mediterranean Region was out of the limit values (5 g/100 g for pine honeys) due to its sucrose content.

Comparison of characteristics of Turkish honeys with honeys from various geographical origins

The identity and quality properties of honey were analysed in different papers from several countries. The quality and authenticity of honey are recognized as essential features from the consumer and producer side worldwide. Table 3 demonstrates a collected study that combines the papers on the physicochemical characteristics of honeys from different countries and overall mean values detected in the presented study. As seen in Table 3, the honeys from different countries exhibited similar values and they meet the international regulations. The physicochemical properties exhibited in this study were in accordance with those reported by Can et al., (2015) for Turkish honeys; except the glucose content which was detected slightly higher in our study. Proline values were only reported for Tunisian honeys and significantly lower than Turkish honeys. Diastase activity and HMF content changed in a wide range among different countries, the highest diastase activity and the lowest values of HMF were reported for Spanish and Brazilian honeys, respectively. Acidity values were similar for all countries; however, the range was noticeably large for honeys from Brazil and Portugal. Moisture contents were similar for all honeys from different countries. Sugar contents vary among the geographical origin. The lowest fructose and glucose contents were reported for honey from Brazil; whereas the highest sugar contents were reported for Egypt, Saudi and Argentina (Table 3).

Table 3. Quality parameters of Turkish honey compared to those reported from other countries.

Country / Honey	Proline (mg/kg)	Diastase activity	HMF (mg/kg)	Acidity (meq/kg)	Moisture (%)	Fructose (%)	Glucose (%)	Sucrose (%)	F+G (%)	F/G	Ref.
Turkey/ Blossom	486.87	10.71	20.22	23.28	16.90	36.85	31.49	0.57	68.34	1.18	PS
Turkey/ Pine	527.34	12.58	15.79	24.87	16.48	33.27	28.04	0.61	61.31	1.19	PS
Turkey/ Blossom	-	6.30-13.20	0-40	-	16-20	32.35 ± 5.65	25.07 ± 6.59	0.91 ± 0.16	54.84-76.18	1.16-2.44	(Can et al., 2015)
Turkey/ Blossom	-	0.10-32.20	0.30-36.50	21.10-47.80	14.50-21.70	31.50-39.10	26.00-34.30	-	57.50-73.40	1.03-1.24	(Güzel and Bahçeci, 2020)
Turkey/ Blossom	117.15-933.49	0.00-20.60	1.10-166.25	2.00-44.00	14.04-18.02	33.89-46.47	26.78-37.35	N.D.-4.10	62.55-77.25	1.03-1.67	(Gürbüz et al., 2020)
Turkey/ Blossom	349-908	10.4-34.9	-	18-29	15.40-18.80	35.51-40.19	26.47-33.70	-	1.10-1.41	-	(Özler et al., 2019)
Palestine/ Blossom	-	-	2.10-34.20	-	14.50-19.00	34.24-41.99	-	1.13-6.94	-	-	(Abdulkhaliq and Swaileh,
Egyptian	-	-	-	-	18.32 ± 0.67	43.30 ± 0.24	26.54 ± 0.31	3.31 ± 0.23	-	1.63 ± 0.05	(El Sohaimy et al., 2015)
Yemeni	-	-	-	-	16.28 ± 0.22	38.76 ± 0.20	25.45 ± 0.22	3.43 ± 0.12	-	1.52 ± 0.04	(El Sohaimy et al., 2015)
Saudi	-	-	-	-	15.64 ± 0.30	50.78 ± 0.41	21.58 ± 0.18	3.59 ± 0.20	-	2.35 ± 0.02	(El Sohaimy et al., 2015)
Portugal/ Blossom	-	3-38	1.75-32.75	17-51.5	13.52-19.7	-	-	-	-	-	(L. R. Silva et al., 2009)
Tunisia	39.62-102.60	-	12.07-27.43	7.11-27.20	-	35.78-37.84	31.07-36.58	N.D.-4.60	-	1.30-1.17	(Boussaid et al., 2018)
Spain	-	11.50-45.80	5.36-15.00	20.10-35.20	15.40-17.38	37.75-41.40	28.80-37.30	0.15-1.43	-	-	(Manzanares et al., 2014)
Morocco	-	6.05-19.10	7.16-30.43	10.69-30.74	14.64-18.59	39.44-42.42	29.25-33.08	0.47-1.86	-	-	(Chakir et al., 2011)
Argentina	-	-	4.00-26.30	9.00-36.8	14.10-18.80	67.70-73.5	-	0.40-5.6	-	-	(Isla et al., 2011)
Brazil	-	10.55-12.40	2.80-7.40	23.60-45.50	17.10-20.50	33.30-38.60	21.00-26.35	0.12-0.50	-	-	(Moreira et al., 2010)
Romania/ Pine	-	-	-	11.80-20.00	14.44-17.20	35.96-40.98	32.98-36.97	-	-	-	(Oroian et al., 2017)
Romania/ Blossom	-	-	-	5.20-37.10	15.43-19.64	33.64-37.65	32.98-36.20	-	-	-	(Oroian et al., 2017)

PS: Presented study; -: Not available data

CONCLUSIONS

The physicochemical analysis results of the honeys produced in various regions of Türkiye reveal a commendable level of quality. The $\delta^{13}C/\delta^{12}C$ isotopic ratio in the majority of samples conforms to established standards, indicating their authenticity. Notably, the low levels of HMF and acidity in most samples not only meet freshness criteria but also underscore the overall quality of the honeys. Furthermore, the moisture content in all samples remains below the 20% threshold set by international regulations, affirming compliance with industry standards. In blossom honeys, glucose and fructose collectively constitute over 60% of the total weight, with only one exception at 58.76%. Pine honeys, on the other hand, exhibit a significant sugar composition, surpassing 45% of the total weight. These findings emphasize the diverse sugar profiles across different honey types. The correlations observed between various quality parameters further strengthen the reliability of the analysis. Significant relationships, such as those between HMF and $\delta^{13}Ch$ and $\delta^{13}Cp$ values; proline and acidity values; and sugar content values, provide robust insights into the overall quality evaluation of different honey types and their regional variations. In conclusion, this comprehensive study not only sheds light on the quality of honeys in distinct regions of Türkiye but also provides a wealth of data encompassing various parameters. The meticulous analysis and correlations between quality indicators contribute to a thorough understanding of the intricate dynamics influencing honey quality across different types and geographical locations.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

Özlem Aslan: Conceptualization, supervision, methodology, investigation, formal analysis, funding acquisition, writing-review and editing. Emine Aytunga Arik Kibar: Methodology, formal analysis, writing-review and editing. All authors read and approved the final manuscript.

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REFERENCES

Abdulkhaliq, A., Swaileh, K. M. (2017). Physico-chemical properties of multi-floral honey from the West Bank, Palestine. *International Journal of Food Properties*, *20*(2), 447–454.

Ahmed, M., Djebli, N., Aissat, S., Khiati, B., Meslem, A., Bacha, S. (2013). In vitro activity of natural honey alone and in combination with curcuma starch against Rhodotorula mucilaginosa in correlation with bioactive compounds and diastase activity. *Asian Pacific Journal of Tropical Biomedicine*, *3*(10), 816–821. https://doi.org/10.1016/S2221-1691(13)60161-6

Al-Khalifa, A. S., Al-Arify, I. A. (1999). Physicochemical characteristics and pollen spectrum of some Saudi honeys. *Food Chemistry*, *67*(1), 21–25. https://doi.org/10.1016/S0308-8146(99)00096-5

Anonymous. (2012). *Turkish Food Codex Communiqué on Honey*.

Bentabol Manzanares, A., García, Z. H., Galdón, B. R., Rodríguez, E. R., Romero, C. D. (2011). Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chemistry*, *126*(2), 664–672. https://doi.org/10.1016/j.foodchem.2010.11.003

Bogdanov, S. (2009). Harmonised Methods of the International IHC. *Bee Product Science, 5*, 1–62. https://doi.org/10.1007/s13398-014-0173-7.2

Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsì, F., Ferrari, G., Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, *11*(2), 265–274.

Can, Z., Yildiz, O., Sahin, H., Turumtay, E. A., Silici, S., Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical

properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, *180*, 133–141.

Cenk ; Küplülü, Ü. (2006). Chemical quality of strained honey consumed in Ankara. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *50*(1), 1–1. https://doi.org/10.1501/Vetfak_0000000058

Chakir, A., Romane, A., Barbagianni, N., Bartoli, D., Ferrazzi, P. (2011). Major and trace elements in different types of Moroccan honeys. *Australian Journal of Basic and Applied Sciences*, *5*(4), 223–231.

Çinar, S.B., Ekşi, A., Coşkun, İ. (2014). Carbon isotope ratio (13C/12C) of pine honey and detection of HFCS adulteration. Food Chemistry, 157, 10–13.

Da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., Fett, R. (2016). Honey: Chemical composition, stability and authenticity. In *Food Chemistry* (Vol. 196, pp. 309–323). https://doi.org/10.1016/j.foodchem.2015.09.051

El Sohaimy, S. A., Masry, S. H. D., Shehata, M. G. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, *60*(2), 279–287.

Elflein, L., Raezke, K.-P., Valkov, V. (2008). Authenticity Testing of Honey. In *World Honeydew Honey Symposium*. https://doi.org/10.1023/A:1025975129938

Escuredo, O., Míguez, M., Fernández-González, M., Carmen Seijo, M. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry*, *138*(2–3), 851–856. https://doi.org/10.1016/j.foodchem.2012.11.015

European Commission. (2002). Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Official Journal of the European Communities*, *L10*, 47–52. https://doi.org/10.1093/jac/dkv056

FAO. (2022). *FAOSTAT Agricultural Production Database, Food and Agriculture Organization of the United Nations, Rome*. FAO, Livestock Information and Policy Branch.

Finola, M. S., Lasagno, M. C., Marioli, J. M. (2007). Microbiological and chemical characterization of honeys from central Argentina. *Food Chemistry*, *100*(4), 1649–1653. https://doi.org/10.1016/j.foodchem.2005.12.046

Golob, T., Plestenjak, A. (1999). Quality of Slovene Honey. *Food Technology and Biotechnology*, *37*(3), 195–201. https://doi.org/10.1002/em.20054

Güney, F. (2020). Physicochemical quality characteristics of Southeastern Anatolia honey, Turkey. International Journal of Analytical Chemistry, 2020.

Gürbüz, S., Çakıcı, N., Mehmetoğlu, S., Atmaca, H., Demir, T., Arıgül Apan, M., Atmaca, Ö. F., Güney, F. (2020). Physicochemical quality characteristics of Southeastern Anatolia honey, Turkey. *International Journal of Analytical Chemistry*, *2020*(1), 8810029.

Güzel, N., Bahçeci, K. S. (2020). Çorum Yöresi̇ Ballarinin Bazi Ki̇myasal Kali̇te Parametreleri̇nin Değerlendi̇ri̇lmesi̇. *Gıda*, *45*(2), 230–241.

Hermosín, I., Chicón, R. M., Cabezudo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry*, *83*(2), 263–268. https://doi.org/10.1016/S0308-8146(03)00089-X

Hernández, O. M., Fraga, J. M. G., Jiménez, A. I., Jiménez, F., Arias, J. J. (2005). Characterization of honey from the Canary Islands: Determination of the mineral content by atomic absorption spectrophotometry. *Food Chemistry*, *93*(3), 449–458. https://doi.org/10.1016/j.foodchem.2004.10.036

Iglesias, M. T., Martín-Álvarez, P. J., Polo, M. C., De Lorenzo, C., González, M., Pueyo, E. (2006). Changes in the free amino acid contents of honeys during storage at ambient temperature. *Journal of Agricultural and Food Chemistry*, *54*(24), 9099–9104. https://doi.org/10.1021/jf061712x

Isla, M. I., Craig, A., Ordoñez, R., Zampini, C., Sayago, J., Bedascarrasbure, E., Alvarez, A., Salomón, V., Maldonado, L. (2011). Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology*, *44*(9), 1922–1930.

Kahraman, T., Buyukunal, S. K., Vural, A., Altunatmaz, S. S. (2010). Physico-chemical properties in honey from different regions of Turkey. *Food Chemistry*, *123*(1), 41–44. https://doi.org/10.1016/j.foodchem.2010.03.123

Karabagias, I. K., Badeka, A., Kontakos, S., Karabournioti, S., Kontominas, M. G. (2014). Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry*, *146*, 548–557. https://doi.org/10.1016/j.foodchem.2013.09.105

Kirs, E., Pall, R., Martverk, K., Laos, K. (2011). Physicochemical and melissopalynological characterization of Estonian summer honeys. *Procedia Food Science*, *1*, 616–624. https://doi.org/10.1016/j.profoo.2011.09.093

Manzanares, A. B., García, Z. H., Galdón, B. R., Rodríguez, E. R., Romero, C. D. (2014). Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *LWT-Food Science and Technology*, *55*(2), 572–578.

Martin, G. I., Macias, E. M., Sanchez, J. S., Rivera, B. G. (1998). Detection of honey adulteration with beet sugar using stable isotope methodology. Food Chemistry, 61, 281–286.

Mateo, R., Bosch-Reig, F. (1997). Sugar profiles of Spanish unifloral honeys. *Food Chemistry*, *60*(1), 33–41. https://doi.org/10.1016/S0308-8146(96)00297-X

Mendes, E., Brojo Proença, E., Ferreira, I. M. P. L. V. O., Ferreira, M. A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers*, *37*(3), 219–223. https://doi.org/10.1016/S0144-8617(98)00063-0

Mohammed, S. E. A. R., Babiker, E. E. (2009). Protein Structure , Physicochemical Properties and Mineral Composition Of Apis Mellifera Honey Samples of Different Floral Origin. *Australian Journal of Basic and Applied Sciences*, *3*(3), 2477–2483.

Moreira, R. F. A., De Maria, C. A. B., Pietroluongo, M., Trugo, L. C. (2010). Chemical changes in the volatile fractions of Brazilian honeys during storage under tropical conditions. *Food Chemistry*, *121*(3), 697–704.

Oroian, M., Ropciuc, S., Paduret, S., Sanduleac, E. T. (2017). Authentication of Romanian honeys based on physicochemical properties, texture and chemometric. *Journal of Food Science and Technology*, *54*, 4240–4250.

Ouchemoukh, S., Louaileche, H., Schweitzer, P. (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food Control*, *18*(1), 52–58. https://doi.org/10.1016/j.foodcont.2005.08.007

Özler, H., Cınbırtoğlu, Ş., Güney, F., Çakıcı, N., Türkarslan, N. (2019). Physicochemical Analysis Of Some Honey Samples From Konya and Karaman Regions. *Arıcılık Araştırma Dergisi*, *11*(1), 1–7.

Padovan, G. J., De Jong, D., Rodrigues, L. P., Marchini, J. S. (2003). Detection of adulteration of commercial honey samples by the13C/12C isotopic ratio. *Food Chemistry*, *82*(4), 633–636. https://doi.org/10.1016/S0308-8146(02)00504-6

Padovan, G. J., Rodrigues, L. P., Leme, I. A., Jong, D. De, Marchini, J. S. (2007). Presence of C4 sugars in honey samples detected by the carbon isotope ratio measured by IRMS. *Eurasian Journal of Analytical Chemistry*, *2*(3), 134–141.

Silva, L. R., Videira, R., Monteiro, A. P., Valentão, P., Andrade, P. B. (2009). Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemical Journal*, *93*(1), 73–77.

Simsek, A., Bilsel, M., Goren, A. C. (2012). 13C/12C pattern of honey from Turkey and determination of adulteration in commercially available honey samples using EA-IRMS. *Food Chemistry*, *130*(4), 1115–1121. https://doi.org/10.1016/j.foodchem.2011.08.017

Singh, I., Singh, S. (2018). Honey moisture reduction and its quality. *Journal of Food Science and Technology*, *55*, 3861–3871.

Thrasyvoulou, A. T. (1986). The use of HMF and diastase as criteria of quality of Greek honey.

Journal of Apicultural Research, *25*(3), 186–195. https://doi.org/10.1080/00218839.1986.11100715

Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., Dogan, M., Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, *46*, 124–131. https://doi.org/10.1016/j.indcrop.2012.12.042

Tosun, M. (2013). Analytical Methods Detection of adulteration in honey samples added various sugar syrups with 13 C/ 12 C isotope ratio analysis method. *Food Chemistry*, *138*, 1629–1632. https://doi.org/10.1016/j.foodchem.2012.11.068

Uçurum, H. Ö., Tepe, Ş., Yeşil, E., Güney, F., Karakuş, S., Kolayli, S., ... Çakici, N. (2023). Characterization of Turkish pine honey according to their geographical origin based on physicochemical parameters and chemometrics. European Food Research and Technology, 249(5), 1317-1327.

Ünal, C., Küplülü, Ö., (2006). Chemical quality of strained honey consumed in Ankara. Ankara Üniversitesi Vet. Fakültesi Derg., 50, 1–1, doi:10.1501/Vetfak_0000000058.

Viuda-Martos, M., Ruiz-Navajas, Y., Zaldivar-Cruz, J. M., Kuri, V., Fernández-López, J., Carbonell-Barrachina, Á. A., Pérez-Álvarez, J. Á. (2010). Aroma profile and physico-chemical properties of artisanal honey from Tabasco, Mexico. *International Journal of Food Science and Technology*, *45*(6), 1111–1118. https://doi.org/10.1111/j.1365-2621.2010.02243.x

Vorlová, L., Čelechovská, O. (2002). Activity of Enzymes and Trace Element Content in Bee Honey. *Acta Veterinaria Brno*, *71*(3), 375–378. https://doi.org/10.2754/avb200271030375

White, J. W., Winters, K. (1989). Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *J. Assoc. Off. Anal. Chem.*, *72*(6), 907–911.

White, J. W., Winters, K., Martin, P., Rossmann, A. (1998). Stable Carbon Isotope Ratio Analysis of Honey: Validation of Internal Standard Procedure for Worldwide Application. *Journal of AOAC International*, *81*(3), 610–619.

Wuensch, K. L., Evans, J. D. (1996). Straightforward Statistics for the Behavioral Sciences. *Journal of the American Statistical Association*, *91*(436), 1750. https://doi.org/10.2307/2291607

Yardibi, M. F., Gumus, T. (2010). Some physico-chemical characteristics of honeys produced from sunflower plant (Helianthus annuus L.). *International Journal of Food Science and Technology*, *45*(4), 707–712. https://doi.org/10.1111/j.1365-2621.2010.02184.x