# Characterization of physical and chemical properties of honey from Northeastern Anatolia of Türkiye

**Research Article**


## ABSTRACT

The aim of this study was to investigate the physical and chemical properties of natural highland honey produced in the Northeastern Anatolia region of Türkiye. In 2020, 24 honey samples collected and sold during the honey harvest season (July and August) were purchased from local vendors. Moisture, color, acidity, pH, conductivity, diastase and invertase activity, C13, C13 protein-honey, C4 analysis and sugar components were analyzed in honey samples. The honey samples were found to contain 17.4% moisture. The mean invertase level were found to be 156.216 U/kg. The freshness and enzymatic activity of the honey were shown by the average diastase number of 12.8 DS. The mineral content and overall purity of the honey was indicated by an electrical conductivity value of 0.17 mS/cm. The average acidity value 15.9 meq/kg, fructose/glucose ratio 1.26% and the average color value 33 mm Pfund determined. The average sugar contents in the honey samples were as follows: fructose 37.4%, glucose 29.5%, sucrose 1.4%, turanose 1.8%, maltose 0.6%, isomaltose 0.2%, erlose 0.3%, trehalose 0% hybriditose 0% maltotriose 0% fructose+glucose. 66.9%, fructose/glucose 1.26, glucose/water 2.0. The mean difference between honey protein and honey delta C13 data is -0.58 and C4 sugar ratio is 0. In conclusion, valuable findings were obtained on the physical and chemical properties of honey from the northeastern Anatolia region of Türkiye. Further research can build on these findings to explore the unique properties and potential benefits of honey from this region and contribute to its value and utilization in various industries.


**Keywords:** Diastase, honey, moisture, invertase, sugar


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## INTRODUCTION

Honey is a natural sweetener with a unique flavor and scent that is made from the nectar of different flowering plants. Its quality and usefulness for various culinary and therapeutic purposes are significantly influenced by its physical and chemical qualities (Thrasyvoulou et al., 2018). For both consumers and producers, it is essential to comprehend these features. Color, viscosity, and texture of honey are indicators of its quality and can affect how desirable it is for culinary usage (Dominguez and Centurión, 2015). As an illustration, lighter-colored honey is frequently chosen for its mild flavor whereas darker honey has a more strong flavor. Viscosity, or thickness, has an impact on the ease of usage and spreadability in cooking and baking.

However, honey's chemical characteristics, such as its moisture content, pH level, and sugar content, can affect both its stability and possible health advantages (da Silva et al., 2016). While honey's pH level impacts its acidity and preservation, its moisture content is crucial for preventing fermentation and rotting (El Sohaimy et al., 2015). Additionally, the distinct chemical makeup of carbohydrates like fructose, glucose, and sucrose influences the flavor of honey.

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On the other hand, the chemical properties of honey, including its moisture content, pH level, and sugar composition, can influence its stability and potential health benefits (da Silva et al., 2016). The moisture content of honey is critical for preventing fermentation and spoilage, while pH level affects its acidity and preservation. Additionally, the unique composition of sugars, such as fructose, glucose, and sucrose, contributes to honey's taste, texture, and nutritional profile (Aparna and Rajalakshmi, 1999).

Understanding these physical and chemical properties allows consumers and producers to evaluate the quality, authenticity, and potential applications of honey. It helps ensure that honey meets the necessary standards for culinary uses, such as baking, sweetening beverages, or drizzling over foods. Characterization of the physical and chemical properties of honey plays a crucial role in ensuring its quality, authenticity, nutritional value and safety for consumers (Solayman et al., 2016). It is also very important to optimize processing methods and packaging to preserve the composition of honey. Understanding the physical properties of honey, such as moisture content and viscosity, is important for determining its storage requirements and shelf life (Eshete and Eshete, 2019**)**. On the other hand, physical and chemical analyses can help identify the geographical and botanical origin of honey. Analyzing chemical properties can reveal if honey has been adulterated with sugars, syrups, or other substances. Furthermore, studying the properties of honey is essential for exploring its potential medicinal applications, as honey has been used in traditional medicine for its antimicrobial and antioxidant properties (Hamadou et al., 2022).

The types and ratios of sugars in honey are referred to as the honey's sugar composition. Fructose, glucose, and sucrose are the three major sugars found in honey. Since glucose and fructose are monosaccharides, they are simple sugars that cannot be divided into smaller components. They make up the majority of the sugar in honey, making up between 85 and 95 percent of its total sugar content (Al-Farsi et al., 2018). Since fructose is a little bit sweeter than glucose, it is what gives honey its distinctive sweetness. The sugar that gives the body energy and is readily taken into the bloodstream is called glucose. Contrarily, sucrose is a disaccharide made up of one glucose and one fructose molecule. It typically accounts for 1% to 5% of the honey's total sugar content (da Silva et al., 2016). Because bees contain enzymes called invertase that break down sucrose into its separate sugar components during the honey-making process, sucrose is less common in honey than fructose and glucose. The flower source of the nectar, climatic circumstances, and bee foraging habits are only a few of the variables that might affect the relative quantities of these sugars in honey (Machado et al., 2018). Because of this, dependent on their sugar makeup, various types of honey can have marginally different tastes, textures, and crystallization tendencies. The sugar content of honey influences its physical characteristics and overall quality in addition to flavoring it. The proportion of fructose to glucose determines the viscosity and crystallization potential of honey. While a higher glucose level encourages quicker crystallization, a higher fructose content makes honey more viscous (Nurul Zaizuliana et al., 2017). Both consumers and producers should be aware of the sugar content in honey. Customers can choose from a variety of honey types to suit their tastes, and producers can examine the sugar composition to make sure the honey satisfies strict quality requirements and maintains its stability throughout storage.

Türkiye has a long history of beekeeping and honey production, and different locations are renowned for their distinctive honey tastes and

properties. Northeastern Türkiye is a location renowned for its varied flora, which helps to produce many kinds of honey. Beekeeping is a significant agricultural industry in Türkiye, which ranks among the top nations in the world for honey production, according to the Turkish Statistical Institute (TSE, 2020). Based on elements including weather, the accessibility of floral sources, and beekeeping techniques, the precise honey production numbers may change from year to year. In this study, it was aimed to examine the physical and chemical properties of natural highland honey produced in northeastern Anatolia of Türkiye region.

## MATERIALS AND METHODS

### *Collection of honey samples*

Following the 2020 honey harvest (July and August), 24 honey samples were purchased from beekeepers who produce and sell honey in Türkiye's Northeastern Anatolia region. The honey samples were collected from Posof (41°30'34.55" N-42° 43' 40.08" E)(n=3), Göle (40° 47' 59.99" N, 42° 36' 59.99" E) (n=5), Hanak (41°14'37.14" N 42°51'6.80" E) (n=2), Damal (41° 20' 26.60"N, 42° 50' 28.28"E)(n=4), Ardahan Province (41° 06' 31.36" N, 42° 42' 7.99" E) (n=6), Olur (40° 49' 59.99" N, 42° 07' 60.00" E)(n=1), and Oltu (40° 32' 26.39" N, 41° 59' 26.39" E) (n=3) regions located in northeastern Anatolia, Türkiye. 400 g of strained honey samples were collected from each producer and stored at room temperature in a dark place until analysis.

### *Moisture content analysis*

The moisture content honey is determined a digital refractometer (Hanna, German), that was regularly calibrated using distilled water or another approved reference material and thermostated at 20°C (Bogdanov and Martin, 2002).

### *Color analysis*

Color analyzes of honey samples were made according to the method based on photometrically reading the color in terms of Pfund Scale (Smetanska et al., 2021).

### *Sugar analysis*

Sugar analysis of the honey samples was carried out using HPLC with a Refractive Index detector. A carbohydrate analysis column (3.9 300 mm) with 10 m-diameter particle size was used for the separation. Throughout the analysis, the column was held at a constant 25°C. Acetonitrile and water made up 80% of the mobile phase. Samples were injected at a flow rate of 2 mL/min in quantities of 25 µL. Peaks in the sample were found by comparing retention times obtained by standards. To confirm the accuracy of the chromatographic peaks, standards were also inserted into the honey samples. Peak quantification was done using average peak areas and duplicate injections. The glucose and maltose standards (Sigma-Aldrich) were used to calculate the sugar content of honey (Bogdanov and Martin, 2002).

### *pH and acidity analysis*

The pH and acidity values of the honey samples were determined using the TSE 3036-2002 technique. A 10% (w/v) solution of honey produced in milli-Q water was tested using a pH meter (Hanna instruments, Italy) (TSE, 2002).

### *Electrical conductivity analysis*

Using a conductivity meter (Meterlab-CDM230, Türkiye), electrical conductivity levels were evaluated in accordance (Bogdanov and Martin, 2002).

### *Diastase activity analysis*

Handling a UV-Spectrophotometer (Shimadzu UV-1800, Japan) allowed diastase analyses of honey samples were determined in accordance with TSE 3036:2002's recommended procedure. The absorbance levels of the samples were determined using a UV-Spectrophotometer (PerkinElmer Lambda 25) in the 600 nm range in order to identify diastase activity (TSE, 2002).

### *Invertase activity analysis*

According to DIN 10754:2002, the invertase activity of honey samples was measured based on the spectrophotometric detection of p-nitrophenol (DIN, 2002).

### *C13, C13 protein-honey, and C4 analysis*

The official methods of AOAC 998.12 were used to determine the C13, C13 protein-honey, and C4 analyses of honey samples (AOAC, 2008).

### *Statistical Analysis*

All of the calculations were performed in triplicate, and the results were provided as mean ± standard deviation. Mean and standard deviation analyses of honey samples were performed with Microsoft Excel.

## RESULTS

The quality of honey is determined by its physical and chemical properties. Determining the properties of honey is very important to know the quality and naturalness of honey. Some of these features are moisture, color, acidity, pH, conductivity, diastase activity, reducing and non-reducing sugar content and invertase activity. Table 1 presented the physical and chemical properties of honey samples.

**Table 1.** Physical and chemical properties of honey samples

| Content | Unit | Mean±Std | Range |
|---|---|---|---|
| Moisture | % | 17.4±1.8 | 14.1 - 18.8 |
| Colour | mm pfund | 33±4.2 | 24.5 - 72.8 |
| pH | meq/kg | 3.6±0.4 | 3.2 - 4.1 |
| Acidity | pH | 15.9±1.6 | 14.2 - 17.8 |
| Invertase activity | U/kg | 156.2±16 | 81.4 - 311.6 |
| Diastase number | DS | 12.8±0.4 | 11.9 - 14.2 |
| Conductivity | mS/cm | 0.17±0.2 | 0.12 - 0.23 |
| C13 Honey | % | -24.91±0.13 | -25.5 - -23.6 |
| C13 Protein/honey | % | -0.58±0.24 | -0.74 - -0.49 |
| C4 | % | 0 | 0 |
| Fructose | % | 37.4±0.9 | 28.8 – 42.4 |
| Glucose | % | 29.5±0.6 | 16.4 - 45.7 |
| Saccarose | % | 1.4±0.2 | 0.7 – 2.3 |
| Turanose | % | 1.8±0.3 | 0.9 – 4.1 |
| Maltose | % | 0.6±0.4 | 0.3 – 1.2 |
| Isomaltose | % | 0.2±0.4 | 0.1 – 0.7 |
| Erlose | % | 0.3±0.1 | 0.2 – 0.5 |
| Trehalose | % | 0 | 0 |
| Melesitose | % | 0 | 0 |
| Maltotriose | % | 0 | 0 |
| Fructose + Glucose | % | 66.9±4.8 | 57.3 – 88.7 |
| Fructose / Glucose | / | 1.26±0.08 | 0.99 – 1.87 |
| Glucose/Water | / | 2.0±0.01 | 1.5 – 2.8 |

## DISCUSSION

In this study, the mean moisture content of honey samples was determined to be 17.4% (Table 1). Honey's moisture content can change depending on where it was made, the climate, how mature it was, and the season (Altun and Aydemir, 2021). The moisture values measured in the honey samples in this study were below the 20% limit determined by the Turkish Food

Codex Honey Communiqué and were determined to be in compliance with the standard (TSE, 2012). According to a study on honey from Algeria, moisture levels in several honey samples ranged from 14% to 18% (Rebiai and Lanez, 2014). Bengü and Kutlu (2018) reported a range of moisture content (14.81-15.91%) in honey produced in Bingöl region of Türkiye. Arıcı and Gökçe (2023) reported the average moisture content of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 15.43±0.06%. The moisture content of Turkish honey determined in this study (14.1%-18.8%) indicates similar moisture content in other studies. It's important to remember that honey's permissible moisture range often falls below 20%. This limit was acknowledged by international honey quality norms by the European Union Council Directive (Council, 2001). If the moisture content of the honey is higher than this, it could ferment or become spoiled. Consequently, the mean moisture content (17.4%) implies an appropriate level within the desired rage for honey quality.

The color value of honey is an important parameter that reflects its visual appearance and can provide insights into its floral source and processing. The color values ranged from 27 to 198 mm Pfund according to a study on honey samples from various floras of Algeria with a mean level of 81 mm Pfund (Rebiai and Lanez, 2014). Depending on the floral source and processing techniques, honey's color may change. Due to its aesthetic appeal and link to softer flavor profiles, lighter-colored honey is frequently favored, while darker-colored honey is generally perceived to have stronger flavors. Direct comparisons are difficult since different countries and areas may use different color grading systems and standards (Dominguez and Centurión, 2015). The average color value of 33 mm Pfund in the honey samples examined for this study, however, points to a moderate color intensity that may be impacted by the distinctive floral sources and beekeeping procedures used in the northeastern Anatolia of Türkiye.

One of the enzymes secreted from the honeybees' cephalic and thoracic glands, invertase has the highest activity when it comes to honey maturation (Al-Sherif et al., 2017). It was found that all samples fit very well with this measurement scale with an average of 156.216 U/kg when the invertase values obtained within the scope of this study are compared with the ratio of IU 73.45 recommended by the International Honey Commission (IHC) in terms of proof and freshness of honey not being heat-treated (Table 1). The amount of invertase and the overall quality of honey are directly correlated. According to our results, honey samples had an average invertase concentration of 156.2 U/kg. Invertase levels in multifloral honey samples ranged from 29.40 – 166.50 U/kg according to a study on honey from different regions of Bulgaria (Manolova et al., 2018). The invertase concentration in this study within this range, indicating a similar enzymatic activity. An essential enzyme called invertase breaks down sucrose into its glucose and fructose molecules, adding to the sweetness and digestibility of honey. Stronger enzymatic activity and a stronger ability to break down sucrose are indicated by higher invertase levels. It is significant to remember that invertase levels might change based on things like the honey's floral source, the surrounding environment, and beekeeping techniques. Therefore, the precise invertase level of 156.216 U/kg in this study offers important insights into the enzymatic activity and product quality.

An enzyme called diastase is naturally present in honey, and its concentration varies depending on the flora's place of origin and the quantity of heat applied (Çiftçi and Parlat, 2018). The Turkish Food Codex Honey Communiqué states that blossom honey must have 8 or more diastases (TFC, 2012). Diastase numbers ranged from 3.99 DS to 49.42 DS,

according to a study on Andalusian honey samples from Spain (Serrano et al., 2007). Honey samples from Northeastern Anatolia of Türkiye had a mean diastase number of 12.8 DS, which is within this range and suggests a similar level of enzymatic activity. Diastase is an enzyme that converts starches, such maltose in honey, into less complex sugars. The honey's diastase activity level is indicated by the honey's diastase number, which might reveal information about the honey's quality. Diastase activity can vary based on the floral source, weather, and beekeeping procedures; it is crucial to keep in mind. Because of this, honeys' from Northeastern Anatolia of Türkiye average diastase number, which is 12.8 DS, tells us a lot about the honey's enzymatic activity and quality. Similar to the findings of our study, Belli (2019) reported that the number of diastases varied between 3.38-13.18 in honey collected in Muğla province of Türkiye. Kara et al., (2022) reported the mean diastase number of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.0-10.9 DS. Bengü and Kutlu (2018) reported diastase number between 16.17-20.61 in honey produced in Bingöl region of Türkiye, which is higher than our findings.

One factor used to differentiate between floral and secretory honeys is electrical conductivity. The electrical conductivity of honey is a measure of its mineral content and can provide insights into its quality and purity. In the honey samples from Northeastern Anatolia of Türkiye which examined in this study, the electrical conductivity is an average level of 0.17 mS/cm, that complies with the Turkish Food Codex Honey Communiqué (TFC, 2012). Electrical conductivity measurements on honey samples from various regions of Vojvodina ranged from 0.08 to 1.99 mS/cm (Živkov-Baloš et al., 2019). Belli (2019) reported that a range of electrical conductivity (0.63-1.67 mS/cm) in honey samples collected in Muğla province of Türkiye. Arıcı and Gökçe (2023) reported the average electrical conductivity of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 0.228±0.001 (mS/cm). Kara et al., (2022) reported the mean electrical conductivity of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.33-0.86 mS/cm. Honey samples from Northeastern Anatolia of Türkiye had a mean electrical conductivity of 0.17 mS/cm, which is within this range and suggests a similar mineral concentration. According to the nectar source and other environmental circumstances, honey with higher electrical conductivity levels may include more minerals. High electrical conductivity, though, can also signal the existence of additional sugars or other impurities.

Honey's acidity value is a crucial indicator of both its pH level and freshness. Depending on the plant source and the region of production, honey's acidity may vary. Honey's total acidity must not exceed more than 50 meq/kg, according to Turkish Food Codex Regulation (TFC, 2012). According to our study, the Turkish Food Codex Honey Communique exceeded the acidity value of honey from the Northeastern Anatolia of Türkiye (14.2-17.8 meq/kg). According to our previous study on samples of honey from Erzurum highland of Türkiye, the acidity ranged from 20.0 meq/kg to 20.8 meq/kg (Altun and Aydemir, 2021). Belli (2019) reported that the acidity value 8.95-27.9 meq/kg in honeysamples collected in Muğla province of Türkiye. Arıcı and Gökçe (2023) reported the mean free acidity values of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 14.584±0.427 meq kg-1. Kara et al., (2022) reported the mean free acidity values of 24 honey samples collected from the center and

districts of Tokat province of Türkiye as 26.0±0.12-48.0±0.16 meq/kg. Honey from Northeastern Anatolia of Türkiye, with an average acidity value of 15.9 meq/kg, is lower than this range, indicating a comparable acidity level. Honey's acidity is mostly caused by the presence of organic acids like gluconic acid. Higher acidity values could be a sign of increasing honey fermentation or deterioration. It is important to note that permitted amounts of acidity in honey might change based on local laws and standards. However, lower acidity values are typically favored as they signify fresher and better honey. Therefore, honey samples of this study had an average acidity value of 15.9 meq/kg indicates a comparatively low acidity level, indicating good quality and freshness.

The fructose/glucose ratio in honey is a characteristic that reveals both the honey's tendency for crystallization and its place of origin. It also reflects the composition and quality of honey. The Turkish Food Codex Honey Communiqué states that honey should have a fructose/glucose ratio of 0.9 to 1.4 (TFC, 2012). The average fructose/glucose ratio of honey samples analyzed in this study was 1.26. Fructose/glucose ratio in polyfloral honey samples of Romania is 1.29 according to a study (Scripca and Amariei, 2018). The honey samples from this study have an average fructose/glucose ratio of 1.26, which falls within this range, indicating a similar composition. The fructose/glucose ratios in a different study on honey samples from Egyptian and Yemeni honey samples ranged from 0.42 to 2.35 (El Sohaimy et al., 2015). Kara et al., (2022) reported the range of fructose/glucose ratio of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.98-2.62. Once more, our study's average fructose/glucose ratio of 1.26% falls within the range that has been previously reported, indicating a comparable composition. The proportion of fructose to glucose in honey is a crucial sign of its floral origin, level of maturity, and level of processing. more fructose/glucose ratios often indicate more fructose content, which contributes to honey's sweetness and stability. They are also correlated with higher honey quality. It's important to remember that fructose/glucose ratios might change based on the type of flower, the area, and the weather. However, the honey samples examined in your study had an average fructose/glucose ratio of 1.26%, which points to a balanced and unique composition.

## CONCLUSION

In conclusion, this study on the physical and chemical properties of honey from northeastern Anatolia of Türkiye provides valuable insights into its quality and characteristics. The findings demonstrate that the honey from Northeastern Anatolia of Türkiye possesses certain specific properties that contribute to its overall quality and potential culinary and medicinal applications.

The honey's 17.4% moisture content was found to be an acceptable level of moisture for honey preservation. The analysis of 156.216 U/kg of invertase suggests the existence of enzymes that contribute to the honey's natural sweetness and digestibility. With a diastase number of 12.8 DS, the honey was found to have enzymes that represent its freshness and enzymatic activity. The honey's mineral content and overall purity can be deduced from its electrical conductivity value of 0.17 mS/cm. Finally, the acidity level of the honey is shown by its average value of 15.9 meq/kg; lower values denote a fresher and higher-quality product. The honey's sweetness and stability are attributed to its balanced composition, which is indicated by the fructose/glucose ratio of 1.26%. The honey samples under study had a moderate level of color intensity, as indicated by the color value of 33 mm Pfund.

These results highlight the quality, authenticity, and potential advantages of honey

from Northeastern Anatolia of Türkiye for a variety of culinary and therapeutic uses while also advancing our understanding of the physical and chemical characteristics of this type of honey.

While the study provides valuable insights into the physical and chemical properties of natural highland honey in the northeastern Anatolia region of Türkiye, there are several limitations that should be considered: a) The relatively small sample size may not fully represent the variability that could exist in honey produced in the region, b) The findings may not be generalizable to honey produced in other cities of this region with different environmental conditions and floral sources, c)The study provides only a snapshot of honey properties in 2020 but a longitudinal analysis over multiple years could reveal variations in honey composition. Considering these limits, it is possible to promote the value and application of the honey of this region by conducting additional studies and analyzes on its special qualities and potential areas of use.

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