Progress in Nutrition 2021; Vol. 23, N. 1: e2021020 DOI: 10.23751/pn.v23i1.9510

Orignal article

Determination of physicochemical properties of raw honey samples

Mehmet Beykaya

Iğdır University, Faculty of Tourism, Department of Gastronomy and Culinary Arts, Iğdır, Turkey - E-mail: mehmet.beykaya@igdir.edu.tr

Summary. Honey is a natural food produced by honey bees from nectar of flowers and the secretion of plants caused by certain insects. It has been well-known for its nutritional and medicinal values since ancient times. In this study, 60 honey samples (cotton, citrus, Astragalus, lavender, jerusalem thorn, flower, cedarwood, pine, chesnut and nigella sativa) were collected from different locations and determined their physicochemical properties like hydroxy methyl furfural (HMF), proline, sugar content, invertase, diastase number, moisture, acidity, color and electric conductivity (EC). In all honey samples, glucose and fructose are present in approximately equal proportions. The color of cotton honey is the lightest one. The acid amounts of honeys were ranged between 13.0-34.0 meq/kg. The proline content of the honey samples used in this study varied between 404.2-881.7 mg/kg and the HMF content was varied between 2.5 mg/kg and 12.3 mg/kg according to honey types. Enzymes are one of the quality criteria for the raw honey. Diastase number of honey samples was determined between 6.35 and 18.6 DN and amount of invertase enzyme ranged from 103.3 to 378.1 U/kg. In this study, physicochemical properties of raw honey samples which were collected from different regions were determined. Thus, this systematic study provides a fundamental knowledge of raw honey quality.

Key words: Raw honey, HMF, invertase, proline

Introduction

Honey is a natural food that is produced by honeybees from nectar of flowers and the secretion of plants caused by certain insects. Honey is consumed as a food since ancient times (1,2). Turkey has the potential to produce abundant flower honey because of rich vegetation. In addition to mixed flower honeys, two types of secretory honey are produced in Turkey as well. These are pine honey produced from the secretions of plant-sucking insects (*Marchalina hellenica*) that feed on pine trees, and oak honey produced from sweets secreted by the sweating of oak trees (3). The composition of honey differs with each other depending on the plant source and climatic conditions (4).

Basically, honey is a condensed mixture of glucose and fructose. Beside these two monosaccharides, there are many carbohydrate derivatives in the composition of honey consisting of disaccharides and oligosaccharides. In addition to these main components, honey contains many different minor components. They can be listed as various minerals, organic acids, phenolic acids, flavonoids, vitamins, enzymes and other proteins (5,6). The composition of honey was effected by the process applied during harvesting or post harvesting, storage conditions as well. In some cases, before packaging some heat treatment could be applied to honey and this treatment could cause the deterioration of some parameters like decreased diastase number and invertase activity, increased HMF content etc. Raw

honey which is not treated in such way could protect its composition (7).

In this study, different types of raw honey samples were collected from different part of Turkey and their physicochemical properties such as hydroxymethylfurfural (HMF), proline, sugar content, invertase, diastase number, moisture, acidity, color and electric conductivity (EC) were investigated.

Material and Method

Chemicals

All reagents and chemicals were used analytical grade from Sigma Chemical Company.

Honey samples

The honey samples were obtained from the experienced beekeepers during the period of 2017-2018 from different locations of Turkey. Melissopalynological characterization was applied (8) (cotton, citrus, atragalus, lavender, jerusalem thorn, multiflower honey, cedarwood, pine, chesnut and *Nigella sativa* were determined) and 60 honey samples were analyzed. Raw honey samples were used in all analyzes, without heating.

Determination of moisture content

Moisture content was determined using a Lega Refractometer HB90. Measured value was corrected according to temperature and expressed as % humidity values (9).

Determination of color

The color of honey samples was determined photometrically according to Pfund Skala (10).

Determination of conductivity

Honey solutions prepared by dissolving each honey in pure water (20% (w/v)) and conductivity values of honey solutions were measured by using EC meters (Electrical conductivity meter). The measured values were converted to 25°C values, the electrical conductivity of honey samples were expressed in 25°C, mS / cm^2 (11).

Determination of Acid Value

10 g of sample was dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker. It was stirred with the magnetic stirrer, phenolftaleyn solution was dropped into the solution. It was titrated with 0.1M NaOH. The volume of color changing was recorded and the acid value was calculated (12).

Determination of sugar content

Sugar content of honey samples were determined by using HPLC (Elite LaChrom, Hitachi, 200/ 4,6 Nucleosil 100-5 NH_2 colone). 5 g of honey was dissolved in 40 mL of pure water without heating then methanol was added into this mixture and filtered. Quantitative and qualitative sugar analyses were performed using the method described by (13). The calibration curves of all analyzed sugars were between 0.994 and 1.000.

Determination of proline

Proline content was determined spectrophotometrically. 5 g of honey sample was dissolved seperately in 50 mL of water and filtered. Filtrate was used for quantification of proline content. Proline stock solution with 0.8 mg/mL concentration was prepared. A concentration range was achieved by serial dilutions. Measurements were conducted triplicate and results were expressed as mean value.

0.5 ml of solution in one tube, 0.5 ml of water (blank test) into a second tube and 0.5 ml of proline standard solution into three other tubes. Then, 1ml of formic acid and 1ml of ninhydrin solution was added to each tube. Cap the tubes carefully and shake vigorously for 15 minutes. Place in a boiling water bath for 15 minutes, immersing the tubes below the level of the solution. Transfer to a water bath at 70°C for 10 minutes. Add 5ml of the 2- propanol-water-solution to each tube and cap immediately. Leave to cool and determine the absorbance 45 minutes after removing from the water bath at 510 nm (11).

Determination of hydroxy methyl furfural (HMF)

Hydroxymethylfurfural (HMF) is determined in a clear, filtered, aqueous honey solution using reverse phase HPLC equipped with UV detection. Calibration curve was prepared using standard HMF (5-hydroxym-

ethlfurfural, Sigma-Aldrich) in aqueous medium and readings were performed at 285 nm using an isocratic program using a reverse phase C18 column LiChroCART ® 250-4 RP (10 µm) for analysis (11). Optimized flow rate 1 mL / min and injection volume 20 µL using 10% methanol-90% water as mobile phase. 2.5 g of honey sample was taken and dissolved in 25 mL of pure water, 50 mL of Karez I and 50 mL of Karez II reagent were added, the prepared solution was passed through a 0.45 µm filter and transferred into vials and injected into the conditioned HPLC system (11).

Determination of Invertase

5.00 g of honey with buffer solution were transfered quantitatively into a 25- ml flask and fill to the mark. 5.0 ml of substrate solution was placed in a test tube in the water bath at 40˚C for 5 minutes before adding the honey solution. 0.50 mL of honey solution (starting time) was added to solution and the mixture was incubated at 40 ˚C. After exactly 20 minutes 0.50 ml of the reaction-terminating solution (3M, pH 9.5 tris- (hydroxymethyl) aminomethane solution) was added and mixed again in a mixer (sample solution). For the blank, 5.0 ml of substrate solution incubated at 40˚C at the same time. After five minutes 0.50 ml of reaction-terminating solution stopper was added the tube, mix well and then 0.50 ml of honey solution was added. A separate blank was prepared and for each honey tested. The solutions were cooled to room temperature as quickly as possible and measured the absorbance of the sample solutions and the blank in cells at 400 nm. The readings were taken after about 15 minutes and in any case within one hour (11).

Determination of Diastase Number

Diastase activity is defined as the amount of enzyme that convert 1% starch to the determined endpoint in one hour at 40°C under experimental conditions. Diastase number was determined according to Turkish honey standard (12). 10 g of honey was dissolved in 40 mL of purified water then pH 5.3 acetate buffer and 0.5 M NaCl solution was added. The tubes were mixed well and incubated in 40 ^{0}C for 60 minutes. After 60 minutes, the mixture was cooled down and 0.1 N iodine solution dropped into test tube. The color was checked and diastase number was calculated.

Results and Discussion

Conductivity value of honey is used to differentiate between secretory honeys and flower honeys. This value varies according to the mineral and acid content of honey. Electrical conductivity value is higher in secretion honeys than flower honeys. According to the Turkish Honey Codex, the electrical conductivity value of secretory honeys should be at least 0.8 mS/cm and flower honeys should be at most 0.8 mS/cm (14). Conductivity values of different types of honey samples were determined as 0.21-1.16 mS/cm (20°C). Conductivity of pine honey which is secretion honey was determined as 0.88 mS/cm (20°C). The data were found to be consistent with the codex data. Conductivity values of honey samples are summarized in Figure 1.

If the pfund value of honey color is less than 8 mm, it is 'water white', 9-17 mm is 'extra white', 18-34 mm is white, 35-50 mm is 'extra light amber', 51-85 mm is 'light' amber 'is classified as' amber' if 86-114 mm, and 'dark amber' if it is greater than 114 mm (15). Color depends on changes in nectar, pollen color and non-enzymatic browning reactions. As a result of this study, it was determined that the color of honey samples changed between 20.0-110 mm Pfund. The color value of chestnut honey was 88.3 mm Pfund. Color values of honey samples are given in Figure 2.

The moisture content of the honey, the storage temperature and the pre-applied heat treatments are among the parameters affecting the quality of the honey. In this study, it was determined that the moisture content of honey samples varied between 15.3-18.1%. It is stated that the moisture content of raw honey samples collected from different regions was below 20% (16). The sum of the free acids, lactones and esters determines the total acidity in honey. Furthermore, honey is an acidic food due to organic acids such as gluconic, butyric, acetic, formic, lactic, succinic, malic, citric and oxalic acids of animal and vegetable origin. In this study, it was found that acid content of honey types were ranged between 13.0-34.0 meq/kg. The moisture and acid values of honey samples are summarized in Figure 3. It is stated that the amount of acid in different honey samples were determined maximally as 36.7 meq / kg (3).

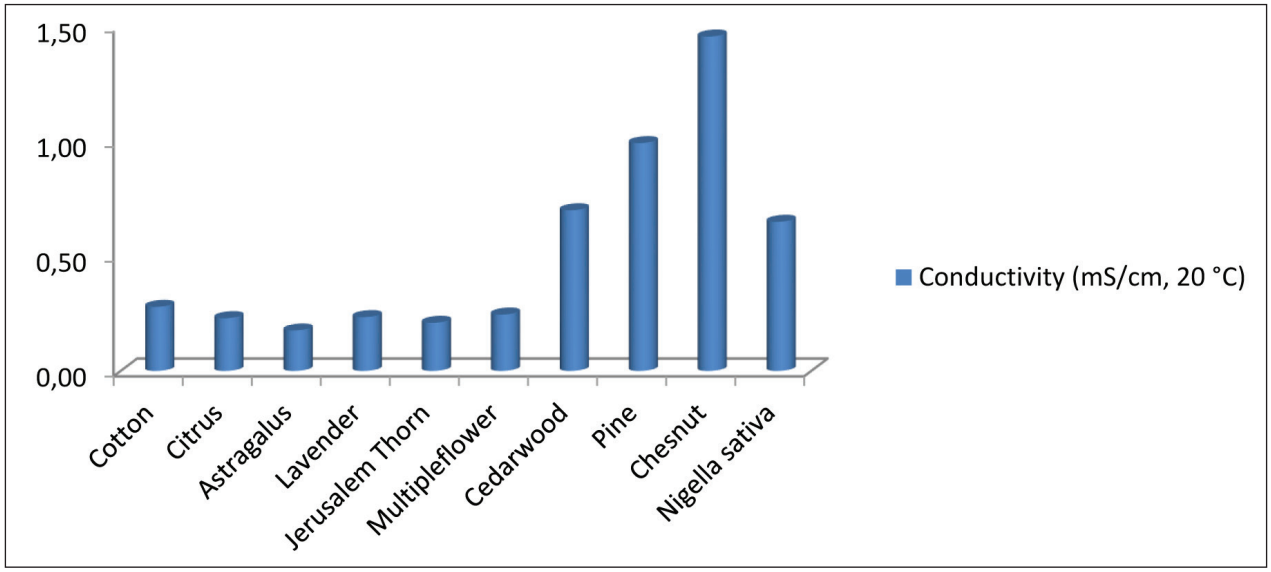

Figure 1. Conductivity of honey samples, The analysis results were obtained in three replicates, since the standard deviation is <0.01, it is not reflected in the figure values

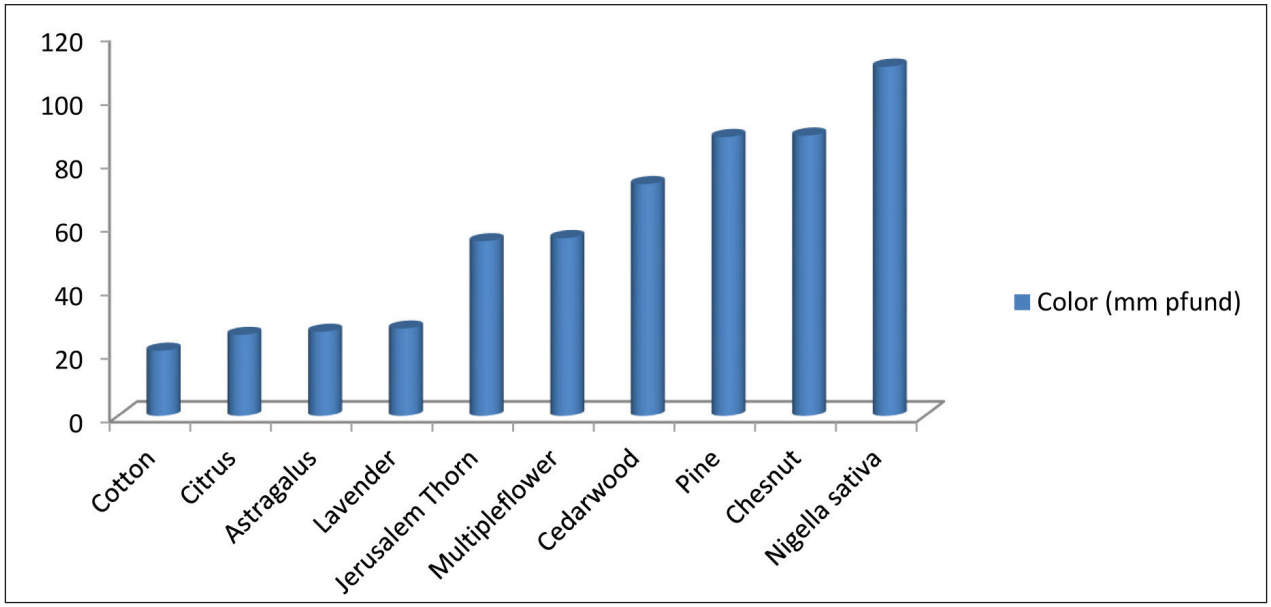

Figure 2. Color of honey samples, The analysis results were obtained in three replicates, since the standard deviation is <0.01, it is not reflected in the figure values

Although the protein source of honey cannot be identified as a nutrient, the amino acids in honey are important for the origin of honey. Proline, lysine, phenylalanine, β-alanine, arginine, serine, glutamic acid and aspartic acid are examples of amino acids found in honey (17). The highest amount of amino acid in honey is proline. Since proline is an amino acid present in plants in various amounts (222 mg / kg in acacia, 956 mg / kg in thyme), the amount of proline is used as a criterion for the separation of honey from bees fed with sugar syrup and honey from nectar (16). The amount of proline recommended by Turkish Codex for honey is minimum 180 mg per kg of honey. It was determined that the proline content of the honey samples used in

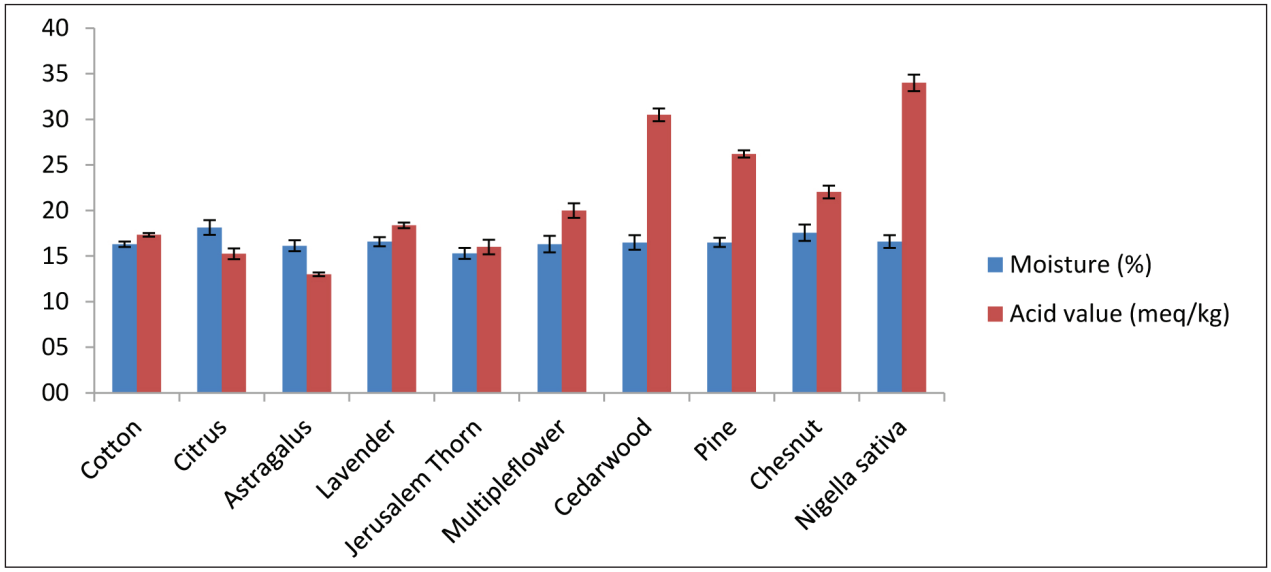

Figure 3. Moisture and acid values of honey samples

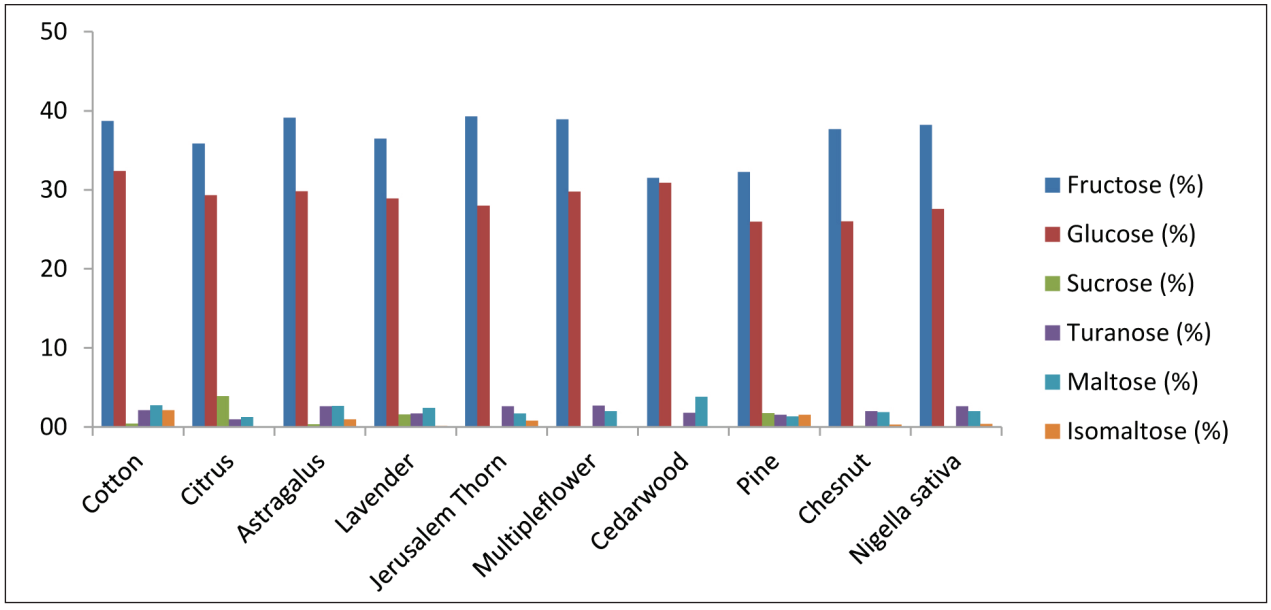

Figure 4. Sugar profiles of honey samples, The analysis results were obtained in three replicates, since the standard deviation is <0.05, it is not reflected in the figure values

the study varied between 404.2-881.7 mg/kg. Proline contents of honey samples are summarized in Table 1. Turkish honey samples were collected from different cities and analyzed. They stated that the amount of proline is changed between 503.46- 696.09 mg / kg (18). It is stated in another study that different types of honey proline content is quite variable and flower honeys proline content changes between 314-890 mg / kg (19).

Enzymes are one of the quality criteria for the raw honey. The main enzymes in honey are diastase, invertase and β-glycosidase. Because of their heat sensitivity, enzymes are used as a determinant of honey quality. The amount of enzymes is quite high in pure and unheated honey. In particular, since the diastase enzyme is heat-resistant, any heat treatment or adulteration in honey can be determined by the reduction

Table 1. Hydroxymethylfurfural, Proline, Diastase and Invertaze content of honey samples

Honey type	Hydroxymethylfurfural HMF (mg/kg)	Proline (mg/kg)	Number of Diastase (DN)	Invertaze (U/kg)
Cotton	2.5±0.07	458.5±20.6	10.1±0.4	171.7±8.6
Citrus	9.1±0.08	300.0±11.8	6.35±0.3	177.5±8.8
Astragalus	6.2±0.05	511.8±21.8	13.2±0.6	144.0±6.9
Lavender	7.1±0.04	570.6±24.2	13.1±0.6	140.7±6.3
Jerusalem Thorn	4.8±0.03	334.3±13.6	9.8±0.5	103.3±4.8
Multiflower Honey	5.5±0.05	542.7±25.4	18.6±0.9	378.1±15.6
Cedarwood	12.3±0.09	404.2±18.2	12.5±0.2	198.5±9.1
Pine	5.0±0.04	678.6±31.6	14.7±0.6	218.8±9.6
Chesnut	5.1±0.04	786.1±33.4	17.5±0.9	233.5±10.8
Nigella sativa	5.8±0.05	881.7±42.6	20.0±0.9	206.6±7.6

in the amount of this enzyme (1). According to the Turkish Food Codex, the number of diastase should be minimum 8. Diastase number of honey samples was determined between 6.35 and 18.6 DN. It was determined that the amount of invertase enzyme ranged from 103.3 to 378.1 U/kg. The amount of invertase in raw honey samples were found to be higher than heat treated honeys. The amounts of enzymes in raw honey according to honey species are summarized in Table 1.

The amount of HMF, a compound released by the breakdown of the fructose present in the composition of honey, is a true criterion for the quality of honey. Because of the heat treatment applied to honey after harvesting, the decomposition of fructose accelerates and the amount of HMF increases. Therefore, the increase in the amount of HMF is an indication that the quality of honey is reduced. HMF should not exceed 40 mg/kg in honey (1,20). In this study, it was determined that HMF amount of raw honey samples varied between 2.5 mg/kg and 12.3 mg/kg according to honey types. The obtained data are summarized in Table 1. It was determined that the data did not exceed the upper limit of the codex.

Another study was conducted to determine the effect of heat treatment and storage conditions on diastase number, invertase number and HMF in honey collected from three different regions of Iraq (4). Average values of physico-chemical properties of fresh honey HMF 3.916 mg/kg, number of diastases 17.66, number of invertases 17.27 and proline 707 mg/kg. The three types of honey were heated at 55, 65, 75°C for 5, 15, 20, 25 min. Honey samples were then taken to room temperature (20-23°C) and stored at this ambient temperature for 26 weeks to evaluate and changes in HMF, diastase number, invertase content were observed. It is stated that the HMF content of honey samples was significantly affected by storage time and heat treatment (21).

Honey is a condensed mixture of two monosaccharides (glucose and fructose) but contains 25 different oligosaccharides. It is also composed of various minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other components (5,6). In honey, glucose and fructose are present in approximately equal proportions. In this study, varied sugar content depending on the honey type was determined. The amount of fructose is between 31.5-39.3%; the amount of glucose was found to be between 26.0-30.9%. Other types of sugar contained in honey species are summarized in Figure 4. It is stated that the fructose content of honey samples belonging to different species varied between 32.35 and 44.12% and the glucose content varied between 17.40 and 25.34% (22). It is reported that physicochemical properties of honey samples has 70% invert sugar, 9 diastase number, 15% moisture, 3 diastase number and 3.83 HMF in average (23.24). It is clear that the results of this study are consistent with the literature.

Conclusion

In this study, physicochemical properties of raw honey samples collected from different regions were

determined. As a result of this study, it was found that the amount of enzymes of different types of raw honey (not heat treated) was quite high. Chestnut honey was found to be rich in proline content compared to other honey types. Multifloral honey was found to be rich in invertase amount and diastase number. It is clear that all data are compatible with codex. Raw honey samples, not heat treated, contain minor elements such as phenolic compounds, amino acids, enzymes etc. that are responsible for the biological activity of raw honey. Honey as a supplementary food is important with these minor elements.

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Correspondence:
Mehmet Beykaya
Iğdır University, Faculty of Tourism, Department of Gastronomy and Culinary Arts, Iğdır, Turkey
E-mail: mehmet.beykaya@igdir.edu.tr