

ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF CHESTNUT HONEYS FROM TURKEY

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ABSTRACT. Thanks to its rich flora, it is possible to produce various monofloral honey in Turkey and one of these honey is chestnut honey. The purpose of this research was to determine the physicochemical and antioxidant properties of chestnut honey collected from different geographical regions of Turkey. The color, humidity, HMF, diastase number, proline, acidity and electrical conductivity values of honey samples were determined as 82.1 mm, 17.33 %, 23.83 mg/kg, 15.12 diastase number, 754.12 mg/kg, 28.68 meq/kg and 0.93 MS/cm respectively. The fructose, glucose and sucrose content in honey samples were determined as 37.09 %, 30.41 and 0.02 respectively, while ratios of other sugars were changed between 0.02 % and 1.99. The total phenolic content of chestnut honey samples was determined as 154.12 mgGAE/100g with Folin Ciocalteu method, while antiradical activities (DPPH method) were found as 37.65 %. There is a need to investigate the biological activities of chestnut honey which has important production potential in Turkey.

1. INTRODUCTION

Honey is a natural, sweet and functional food that meets the energy needs of the human body. The chemical content of honey depends on the type of bees as well as the botanical and geographical origin [1]. According to the source is obtained, honey types are classified under two groups; flower honey and honeydew honey. The honey made by honey bees from the nectars of various flowers is the flower honey. Cotton, chestnut, citrus, astragalus, trifolium, acacia, thyme, sunflower honey types are included in the flower honey group [2]. Carbohydrates, water, organic acids, minerals, enzymes, vitamins, aromatic substances and antioxidants constitute the main components of honey [3]. There are basic monosaccharides such as glucose and fructose which are among the energy-giving carbohydrates as well as 25 different oligosaccharides such as panose, melezitose, and raffinose [4]. Although proteins are not high in honey, the amino acids in honey are important for the origin of honey. Proline, lysine, phenylalanine, γ -amino butyric acid, β -alanine, arginine, glutamine, serine, glutamic acid and aspartic acid are among the amino acids that exist in honey [5]. In addition, honey has many beneficial effects

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in terms of health and one of them is the activity of antioxidants. Honey's antioxidant activity is attributed to phenolic substances. The botanical origin of honey has the greatest influence on its antioxidant activity, while processing, handling and storage affect honey antioxidant activity [6].

Chestnut honey is widely produced in Turkey, especially in Marmara and Western Black Sea regions. Among the monofloral honey produced in the world is the most delicious and highest quality honey. It is known that it has floral, woody, spicy, floral and fruit-specific taste as its sensory characteristics [7]. Chestnut honey is a honey that has dark (amber) color, sharp taste and distinctive intense aroma that leaves a slight burning effect on the throat after it is eaten. Compounds responsible for the taste and aroma of chestnut honey; 1-phenylethanol, cinnamyl alcohol, p-hydroxyacetophenone and aminoacetophenone compounds responsible for biological activity are phenolic acids, such as caffeic acid, ferulic acid and p-coumaric acid [8]. This honey, which is rich in K, Ca and Mg, was found to be high in diastase enzyme activity than other monofloral honey [9, 10]. Dark-colored chestnut honey has high pH, electrical conductivity and ash value. This honey is characterized by a high fructose low glucose content. Because the F/G ratio is high, G/water ratio is low, it crystallizes late [11]. In addition, there are many researches on the biological effects of chestnut honey. Perna et al. [12] reported that chestnut honey had higher antioxidant activity because it contained higher phenolic content, flavonoids and vitamin C than other monofloral honey. Chestnut honey has antimicrobial effect against pathogenic bacteria such as *Erwinia carotowora*, *Yersinia enterocolitica* and *Aeromonas hydrophila* as well as bacteria such as *S. aureus* and *E.coli* [13]. Furthermore, the effect of anti-inflammatory, wound healing and acetylcholinesterase (AChE) inhibitor has been reported [14, 15].

In this study, it was aimed to determine the physicochemical properties, sugar profile and antioxidant activity of chestnut honey with significant production potential and commercial value in Turkey.

2. MATERIALS AND METHODS

a. Physicochemical analysis of honey samples

Chestnut honey samples are obtained from the provinces where Turkey's chestnut honey production is the most. Twenty honey samples were collected in accordance

with the method specified by the Turkish Food Codex Regulation and labeled with harvest date, botanical and geographical origin from Zonguldak, Bursa and Yalova cities in 2016.

Color, moisture, HMF, proline, diastase, proline, acidity, electrical conductivity and sugar analysis were performed according to AOAC directives [16].

b. Melissopalynological Analysis

The palynological analysis of honey samples was carried out according to Louveaux's method [17]. Palynological analyzes showed that more than 80 % of the samples contained chestnut pollen.

c. Chemicals and Biochemical Analysis of Honey Samples

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 3,4,5-Trihydroxybenzoic acid (gallic acid; GA), Folin–Ciocalteu reagent, ascorbic acid (AA) and ethanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The sulfuric acid (H_2SO_4), sodium phosphate (Na_3PO_4), ammonium molybdate ($(NH_4)_2MoO_4$), sodium carbonate (Na_2CO_3) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). The color value of the honey was determined using a Hunter spectrometer (CR-400, Minolta, Osaka, Japan). Moisture content was measured using a refractometer (Atago, Tokyo, Japan), electrical conductivities with a conductometer (WTW inoLab Cond/720, Germany) and optical activity or rotation with a polarimeter (Beta PPP7, England). Sugar analysis of the samples was performed using a refractive detector (RID) with HPLC (Elite La Chrom, Hitachi, Japan) and a reverse phase–amide column (200/4.6 Nucleosil 100-5 NH₂). Quantitative and qualitative sugar analyses were performed using the method described before. The calibration curves of all analyzed sugars were between 0.994 and 1.000. All the analyses were carried out according to the principles of EU legislation [18].

2.4. HPLC-RID Analysis for Sugar profile of honey samples

HPLC-RID analyses were performed by Hitachi, LaChrom Elite® (Hitachi High Technologies America, Inc., San Jose, California) equipped with RI detector (Hitachi, L-2455 Diode Array Detector). HPLC-RID analyses were performed on a reverse phase NH₂ column (200 mm × 4.6 mm id, 5 μm particle; Nucleosil).

Fructose, glucose, sucrose, turanose, maltose, theralose, isomaltose, erlose, melezitose, and maltotriose were determined and normalization calibration method was used [19]. Mobile phase was applied as an isocratic elution; 79-21% acetonitrile/water mixture. Injection volume was 25 μ L, column temperature was 80°C and flow rate was 1.5 mL/min. For sample analysis, about 1 g honey was dissolved with 10 mL ultra-pure distilled water and the solutions were filtered by 0.45 μ m filter (Sartorius, Goettingen, Germany)

2.5. Determination of total phenolic content of honey samples

TPCs were determined using the Folin-Ciocalteu procedure with gallic acid as standard [20]. Briefly, 0.2 mL extract was mixed 1.8 mL distilled water and 1 mL of 0.2 N Folin-Ciocalteu reagent and the contents were vortexed. After 3-min incubation, 1.5 mL of 2% Na₂CO₃ (w/V) solution was added. After vortexing, the mixture was incubated with intermittent shaking for 2 h at room temperature. Absorbance was measured at 760 nm and TPC concentration was calculated as mg of gallic acid equivalents per gram of 100 g sample, using a standard calibration graph.

2.6. Determination of radical scavenging activity (DPPH)

Scavenging activities of the honey samples toward DPPH radical were assessed by using the method described by Molyneux [21] with a minor modification. Briefly, various concentrations of 0.200 mL of extracts of honey were mixed with 3.9 mL of 0.1 mM of DPPH in methanol. The setup was left in the dark for 30 min, at room temperature for allowing to react with stable free radical. After incubation period, the decrease in absorbance at 517 nm was measured spectrophotometrically against control, using a UV-Visible spectrophotometer. The inhibitory effect of DPPH was calculated according to the following formula:

$$\% \text{ Inhibition} = [1 - (\text{Abs}_{\text{Sample}} / \text{Abs}_{\text{Control}})] \times 100$$

Statistical Analysis

SPSS 22.00 package programme was used in statistical analyses. Data was presented as arithmetic means and standard deviations.

3. RESULTS AND DISCUSSION

The physico-chemical analysis of honey samples summarized in Table 1. Colour is one of the important parameters to determine the Botanical origin of honey. In this research, the color of chestnut honey was determined as light amber [22]. It is known that dark-colored honey contains more minerals and has higher phenolic content. The color value obtained was also consistent with a study conducted with 62 chestnut honeys [1].

TABLE 1. Physicochemical properties of chestnut honeys.

| Parameter | Mean \pm SD | Parameter | Mean \pm SD |
|-----------------------|-------------------|-------------------------------|--------------------|
| Colour (Pfund) | 81.82 \pm 6.85 | Proline | 740.00 \pm 94.44 |
| Moisture % | 17.31 \pm 0.83 | Acidity meq/kg | 28.87 \pm 4.78 |
| HMF (mg/kg) | 22.38 \pm 17.55 | Electrical conductivity MS/cm | 0.89 \pm 0.36 |
| Diastase number (DIN) | 15.65 \pm 3.60 | | |

Moisture in honey is one of the most important quality parameters. The moisture content of honey may vary according to botanical origin, climatic conditions, season and honey processing methods. Honeybees do not cap off the comb under normal conditions without falling below 18 % of the moisture value of honey in the comb. However, if the bees harvest the honey without being fully cap off during the harvest, the humidity of the honey will be high. High humidity content in honey causes the crystallization and fermentation of honey. Therefore, the high moisture content in honey is an undesirable property. The moisture content of chestnut honey was found to be 17.31 % on average. Devillers et al. [1] investigated with 469 mono-floral honey samples and their quality data and found the mean of moisture values as 17.60 %.

TABLE 2. Sugar profile of chestnut honeys.

| Sugar | % Mean ±SD | Sugar | % Mean ±SD |
|--------------|-------------------|------------------------|-------------------|
| Fructose | 37.09±2.29 | Erllose | 0.13±0.13 |
| Glucose | 30.41±4.08 | Melesitose | 0.02±0.06 |
| Sucrose | 0.02±0.06 | Maltotriose | 0.00±0.00 |
| Turanose | 1.38±0.38 | Fructose+Glucose (DIN) | 67.50±6.12 |
| | | | |
| Maltose | 1.99±0.28 | Fructose/Glucose | 1.23±0.12 |
| Trehalose | 0.00±0.00 | Glucose/Water (DIN) | 1.76±0.29 |
| Isomaltose | 0.68±0.51 | Total disaccharides | 7.29±2.66 |

The indication of the wrong procedures performed by honey can be understood with honey diastase activity and HMF content. If honey is stored for a long time or exposed directly to heat, the HMF value increases and diastase enzyme activity decreases. These two parameters are important in the quality of honey. The results obtained in this study for HMF (22.38 mg/kg) and diastase activities (15.65) are consistent with Turkey and world honey Codex values [23, 24].

Proline is an amino acid and honey is passed through the saliva during honey processing. It is an important indicator of whether or not honey is associated. In this research, the proline value obtained from chestnut honey is a high value (740 mg/kg). This result shows that the honey being tested is natural and mature. Researchers have determined the proline value of 43 honey, 6 of which are chestnut honey, between 590-609 mg proline /kg honey.

Honey is usually responsible for the taste characteristics while the acidification of honey varies according to the Botanical origin. Electrical conductivity is an analysis used to determine the source of honey. The result obtained in this study for acidity (28.87 meq/kg) is consistent with Turkey and world honey Codex values [23, 24]. Electrical conductivity value obtained was also consistent with a study conducted with Saric et al. [25]; 0.538-1.38 mS/cm.

In this study, fructose content, which is the basic sugars of chestnut, was found to be 37.09 %, glucose content was 30.41 % and saccharose content was 0.02 %. However, turanose, maltose, isomaltose, erlose, melesitose were among the other identified sugars. The contents of the total disaccharides were determined as 7.29 %. Among these sugars maltose was detected at the highest rate. In addition, Fructose+Glucose content, which is important parameters in crystallization, was found to be 67.50, Fructose/glucose content was found to be 1.23 and glucose/water content was found to be 1.76. Chestnut honey is a late crystallized honey. Crystallization is a significant parameter for the market value of honey. In temperate climates, honey can crystallize even under normal storage temperatures and crystallization negatively influences consumer preferences. The majority of honeys are supersaturated solutions with glucose and this glucose can spontaneously crystallize into glucose-monohydrate at room temperature [26]. The crystallization trend of honey from different botanical origins is closely related to some physical and chemical parameters, some of these parameters are glucose, glucose/water, glucose-water/fructose, fructose/glucose ratios and melezitose content. Specifically, honey crystallizes faster when the glucose content is >28–30 %, glucose/water ratio is ≥ 2.1 , fructose/glucose ratio is <1.14 and melezitose ratio is over 10 % [27]. Beside these parameters, the existence of dust, pollen, comb and propolis particles in honey also influences the crystallization of honey [1, 28]. Furthermore, botanical origin, processing conditions, storage conditions, storage temperature, relative humidity and the container in which the honey is kept also influence the crystallization of honey [29].

One of the most important activities of phenolic compounds is antioxidant activity [30]. Honey contains not only phenolic compounds, but also other enzymatic and nonenzymatic compounds that promote antioxidant activity.

Phenolic compounds exert their beneficial health effects mainly through their antioxidant activity and also honey is known to be rich in both enzymatic and non-enzymatic antioxidants. Antioxidant capacity of honey depends on the floral source, seasonal and environmental factors [31]. The total phenolic content of the samples were found between 122.93-174.19 mg GAE/100 in this study. These values are higher than those obtained in previous studies [12, 32, 33] In this research, the antioxidant activity of honey samples tested were determined between 27.52-43.13%. Sagdic et al. [34] and Perna et al. [12] reported the inhibition level of authentic chestnut honeys were $67.92\% \pm 8.58$ and $75.37\% \pm 7.87$, respectively.

In the present study, the physico-chemical properties sugar profile and antioxidant activity of chestnut honey were investigated. The results obtained from this research will give an overview of the properties of chestnut honey which has an important production potential in our country. The results obtained from this research will give an overview of the properties of chestnut honey which has an important production potential in our country. In addition, it provides data for use in the field of health because it has a high level of total phenolic content and antioxidant activity.

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