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Bioactive and Physicochemical Properties of Anatolian Honeys

Sibel Silici[a,b*], Sema Yilmaztürk[c]

[a] *Erciyes University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kayseri, TÜRKİYE*

[b] *Nutral Therapy Co. Erciyes Technopark Kayseri, TÜRKİYE*

[c] *Erciyes University, Graduate School of Natural and Applied Sciences, Agricultural Biotechnology, Kayseri, TÜRKİYE*

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Corresponding Author: Sibel Silici, E-mail: sibelsilici@gmail.com
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ABSTRACT

Determination of physicochemical and biological properties of honeys produced in our country is important both scientifically and economically. Therefore, in this study, some physicochemical and bioactive properties of monofloral honeys, which are produced in our country and have commercial importance, were analyzed. Nine honey samples, having eight monofloral (lavender, chestnut, pine, sunflower, citrus, polyfloral, cotton, linden, and thyme) and one polyfloral honey, were used in the study. Physicochemical parameters such as moisture, *HMF* (5-hydroxymethyl-furan-2-carbaldehyde), diastase and invertase activity, proline value, total acidity, sugar, and bioactivity values, such as *total phenolic content (TPC),* antioxidant and antiradical activity, were characterized for the honey samples. According to the results obtained, the moisture of honey samples (15.8-17.2%), HMF (1.8-22.3 mg/kg), diastase (3.6-13.6) invertase (26.8-265.6 U/kg), proline (290.8-751.2 mg/kg), total acidity (11.6-27.2 meq/kg), fructose (32.6-39.8%), glucose (25.7-33.4%) and sucrose (0.2-2.2%) values were determined. Honey samples were evaluated by the Folin-Ciocalteu method (total phenolic content), phosphomolybdenum test (antioxidant activity), and DPPH (antiradical activity) test. TPC was determined by the Folin Ciocalteu method (25.5-63.42mg GAE/100g), and the highest total phenolic content was observed in thyme honey, followed by pine and chestnut honey. Physicochemical values of honey samples, except diastase activity, were determined within legal Codex limits.

Keywords: Honey, Physicochemical, Bioactivity, HMF, Diastase

1. Introduction

Honey is a honeybee product collected by the honeybee from plant nectars or secretions of insects living on some plants, combined with their own enzymes, and stored in the honeycomb by changing its chemical structure (Anonymous 2020). The physicochemical properties of honey, such as colour, taste, water activity, sugar, protein, phenolic and volatile content, and its bioactivity vary depending on the plant source from which the nectar is collected. Honey can be floral or honeydew honey depending on the source from which it is obtained (Bogdanov 2004). While flower honey is obtained from the nectar of flowers, honeydew is obtained from the digestive waste of the *Homophlebus helenicus* species of insects that commonly live on trees such as *Pinus brutia* in our country. Carbohydrates constitute 82-85% of the dry matter of honey and the most abundant sugars are glucose and fructose (Crane 1976). The taste and color of honey may vary depending on the plant source from which the nectar is collected, for example, honey with a high fructose content is sweeter than honey with a high glucose content. In addition to glucose and fructose, honey contains at least 12 disaccharides such as sucrose, turanose, maltose, and isomaltose (Özkök et al. 2014). When researchers compared the sugar content of honeydew honeys with flower honeys, they reported that trisaccharides such as hybridose and raffinose were higher in honeydew honeys (Bogdanov 2004). Nitrogen (0.4%), which is quite low in honey, comprises 40-65% of natural proteins, while the rest are free amino acids (Arcot & Brand-Miller 2005). Diastase is an enzyme that determines the quality of honey. This enzyme is an indicator of whether the honey has undergone heat treatment. Another enzymes determined in honey, invertase and glucose oxidase. Glucose oxidase, which enables the conversion of glucose to gluconolactone, is responsible for the formation of gluconic acid. Researchers have determined that honeybee saliva contains significant amounts of amylase and glucose oxidase (Arcot & Brand-Miller 2005). Organic acids that determine the characteristic taste of honey are up to 0.5% of its dry matter (Crane 1976). The acidity level of honey is a feature that provides stability against microorganisms.

The vitamin and mineral content of honey is not high. The vitamins and minerals found in honey can only meet 10% of the daily recommended vitamin and mineral intake (Arcot & Brand-Miller 2005). The vitamin and mineral content of honey also varies depending on the content of the collected nectar. Although present in trace amounts, selenium, chromium, and manganese

play a significant role in the nutrition of children (1-15 years of age). The moisture content of honey is usually between 18-20%. The water activity of honey is between 0.5 and 0.6, which is not a level where most bacteria and fungi can survive. Both the low moisture content and high osmotic pressure of honey prevent bacterial growth (Arcot & Brand-Miller 2005).

Consumers are interested in the glycaemic index of honey, and this value varies depending on the plant from which is obtained (Soylu et al. 2015). Since ancient times, people have used honey for therapeutic purposes. Some of these are stimulating the immune system, wound healing properties, and protective properties against cancer (Bogdanov et al. 2008). Honey has antioxidant activity, and this activity varies according to the plant from which the nectar is collected, the content of the nectar, and the season (Lachman et al. 2010). The compounds that provide antioxidant activity of honey are mainly flavonoids (pinocembrin, apigenin, galangin, pinobanksin, kaempferol, luteolin, hesperetin, etc.) and phenolic acids, vitamins, carotenoids, enzymes, organic acids, and amino acids (Aljadi & Kamuriddin 2004; Bertocelj et al. 2007; Alvarez-Suarez et al. 2010). Determination of the physicochemical and bioactive properties of these honeys is important both scientifically and economically. Therefore, this study aimed to investigate some physicochemical and bioactive properties of monofloral honeys produced in our country and of commercial importance.

2. Material and Methods

2.1. Honey samples

Honey samples were obtained from Nutral Therapy Company (Erciyes University Technopark, Kayseri) and beekeepers in 50-100 g glass packages with the harvest date recorded. After the pollen analysis of the honeys stored at room temperature, dominant pollen-containing honeys (monofloral honeys) and a polyfloral honey were included in the study.

2.2. Pollen analysis

Honey samples were named according to melissopalynologic characterization. The method suggested by Louveaux et al. (1978) was followed. Calculations were made after at least 1000 pollen grains were counted in 4 slides taken from the sample, and other analyses were carried out after confirming that the honeys were monofloral. Honeydew honey is characterised by the presence of honeydew elements (HDE), such as microalgae, fungal mycelia, and spores. According to Louveaux et al. (1978), a honey sample is classified honeydew honey if its ratio of HDE/P (P: total pollen content) > 3.

2.3. Physicochemical analyses

All chemicals and reagents used in the analyses were of analytical grade and purchased from Merck (Darmstadt, Germany). All tests were performed in triplicate. The moisture values of honey samples were measured by refractometer (RHB-32 ATC 0-32). HMF was measured by HPLC-UV using the C18 LiChroCART-250-4 RP column (Jeuring & Kupper 1980). Proline content was measured spectrophotometrically (Ough 1969), and enzyme activities were performed according to AOAC (2000). It was measured in Shade units, which are the same as Gothe units. The number DN stands for g starch hydrolysed × h^{-1} at 40 °C per 100 g honey (Bogdanov et al. 2009). Sugar analyses were performed according to Jahanbin et al (2012). HPLC (Agilent 1100, USA), a carbohydrate column (5 µm and 4.6 mm x 250 mm), and a refractive index detector (RID) were used for sugar analysis. Total acidity levels of honey were determined by the titrimetric method according to AOAC (2000).

2.4. Total phenolic content (TPC)

TPC of honeys was determined according to the Folin Ciocalteu method (Singleton & Rossi 1965). Honey samples were weighed into test tubes (1 g) and diluted by adding pure water to obtain an appropriate absorbance in the range of 0.0-1.0. Then, they were homogenized. One mL of diluted reagent was added to the samples. After homogenization, 2 mL of Na_2CO_3 was added at 15-second intervals, the tubes were closed and mixed, and incubated. Absorbances were measured at 765 nm against a blank solution prepared using a spectrophotometer (Varian, Carry 100, UV-Visible, Canada). Results are shown as mg gallic acid equivalent (GAE)/100 g honey.

2.5. Antioxidant activity of honey samples

The phosphomolibdenum method (Prieto et al. 1999) measured the antioxidant activities of honeys and and expressed according to the activity of ascorbic acid. Briefly, 0.4 ml of honey sample was mixed with 4 ml of reagent solution. The absorbance was measured at 695 nm. The antioxidant activity of honeys was calculated as ascorbic acid equivalents (AAE) (mg/1g methanol extract).

2.6. Antiradical activity of honey samples

Antiradical activity of honey samples against DPPH(1,1-dihenyl-2-picrylhydrazyl) radical was determined according to Gyamfi et al. (1999). Briefly, one gram of weighed honey sample was dissolved in methanol (4 mL) and filtered. 50 µL of honey sample

taken from the mixture was mixed with Tris-HCL and DPPH. The absorbance of the samples kept at room temperature for 2 h was measured at 517 nm. The antiradical activity (%) of the samples was calculated.

2.7. Statistical analysis

The results were expressed as mean values±standard deviation (SD). The inter group comparison was done using a one- way ANOVA test, and *post hoc* Tukey tests were used to carry out paired comparisons, p value of<0.05 was considered statistically significant.

3. Results

As a result of pollen analysis of lavender, chestnut, sunflower, citrus, cotton, linden, and thyme honeys, dominant pollen percentages were found to be 46, 92, 42, 18, 46, 48, and 62, respectively. HDE/P ratio of pine honey was determined to be >3. The polyfloral honey sample did not contains any dominant pollen.

Table 1 provides the moisture, *HMF*, enzyme activities (diastase, invertase), sugar compositions (sucrose, fructose, glucose), proline, and total acidity levels of the honey samples. Honey samples showed statistically significant differences in terms of other analysed parameters except glucose and fructose content (P<0.05). Accordingly, the moisture content of the honey samples was determined in the range of 15.7%–17.2%, while the highest was determined in sunflower honey and the lowest in linden honey. We found the *HMF* content of the samples in the range of 1.8-22.3 mg kg^{-1}, with thyme honey having the highest value. Sunflower honey had the lowest *HMF* content. We determined the diastase number (DN) of the honeys within the range of 3.6-19.2 DN. The invertase enzyme activities of the honey samples were in the range of 26.8 to 265.6 U/kg. Invertase enzyme activity was higher in chestnut honey than in other honeys. The total acidity levels of our samples were in the range of 11.6-27 meqg/k. Chestnut honey had the highest value compared to other honeys, while thyme honey had the lowest end value. We determined the fructose content of the honey samples to be between 32.6-39.8% and the glucose content between 25.7-33.4%. The glucose level was higher in polyfloral honey than in other honeys. The sucrose content of the honeys was determined in the range of 0.2-2.2%. Thyme and cotton honeys had the highest sucrose content while chestnut honey had the lowest.

Table 1- Physicochemical properties of honey samples

Honey	***Moisture (%)***	***HMF (mg kg^{-1})***	***Diastase (DN)^{a,b}***	***Invertase (U/kg)***	***Proline (mg kg^{-1})***	***Acidity (meq/kg)***	***Fructose (%)***	***Glucose (%)***	***Sucrose (%)***
Lavender	16.5±0.2^{b}	3.2±0.2^{b}	12.7±0.3^{d}	176.3±8.6^{e}	485±10.2^{e}	21±2.3^{ab}	34.6±4.2	28.8±6.8	1.2±0.1^{b}
Chestnut	16.9±0.2^{bc}	4.4±0.2^{c}	19.2±0.6^{e}	265.6±11.2^{g}	727.6±12.2^{f}	27±2.3^{b}	37±3.4	26.3±6.5	0.2±0.1^{a}
Pine	16.5±0.3^{b}	4.5±0.1^{c}	13.6±0.4^{d}	229.5±9.7^{f}	751.2±12.2^{f}	24±2.4^{b}	32.6±4.2	26.8±6.3	1.9±0.2^{c}
Sunflower	17.2±0.1^{c}	1.8±0.1^{a}	9.9±0.3^{c}	234.0±11.2^{f}	392±10.2^{b}	18±4.2^{ab}	39.8±4.8	30.2±7.2	1.4±0.1^{b}
Citrus	17.1±0.3^{c}	4.2±0.1^{c}	3.6±0.2^{a}	87.8±6.2^{c}	290.8±11.2^{a}	11.6±6.2^{a}	35.6±5.2	29.6±7.4	1.9±0.1^{c}
Cotton	16.4±0.2^{b}	2.2±0.2^{a}	8.6±0.2^{b}	164.6±8.4^{de}	407.7±12.3^{bc}	16±4.2^{ab}	38.6±3.2	32.4±6.2	2.2±0.1^{c}
Polyfloral	15.8±0.1^{a}	14.5±0.1^{d}	9.1±0.4^{bc}	147.2±6.4^{d}	398.9±11.2^{bc}	16,2±4.6^{ab}	36.5±4.2	33.4±5.4	1.2±0.2^{b}
Linden	15.7±0.2^{a}	16.6±0.1^{f}	9.2±0.6^{bc}	61.8±4.2^{b}	423.2±8.6^{cd}	22±5.6^{ab}	37.2±3.2	26.2±4.8	1.4±0.1^{b}
Thyme	16.4±0.1^{b}	22.3±0.2^{e}	8.8±0.4^{bc}	26.8±2.3^{a}	452.6±6.8^{d}	21±4.2^{ab}	36.7±4.2	25.7±4.8	2.2±0.1^{c}
P	0.000	0.000	0.000	0.000	0.000	0.01	0.619	0.774	0.000

The values are expressed as means (standard deviation) of three replicates. Mean values with different letters within a row are significantly different according to Tukey test. [a] Expressed as mean of three replications plus standard deviation. [b] Shade number, corresponds with Gothe number, or g starch hydrolysed h^{-1} at 40 ^{o}C per 100 g honey.

Proline values were found in the range of 290.8-751.2 mg kg^{-1}, with the highest proline being found in pine honey and the lowest in citrus honey. *TPC* values of the honeys were determined in the range of 25.5-63.4 mgGAE/100g. Therefore, we found that thyme honey had the highest value, while citrus honey had the lowest.

The antioxidant activity values of the honey samples were determined in the range of 12.2-42.2 AAEmg/mL. According to the results, the antioxidant activity ranking of the honeys from highest to lowest value is as follows; cotton, sunflower, citrus, pine, thyme, polyfloral, lavender, linden, and chestnut (Figure 1)

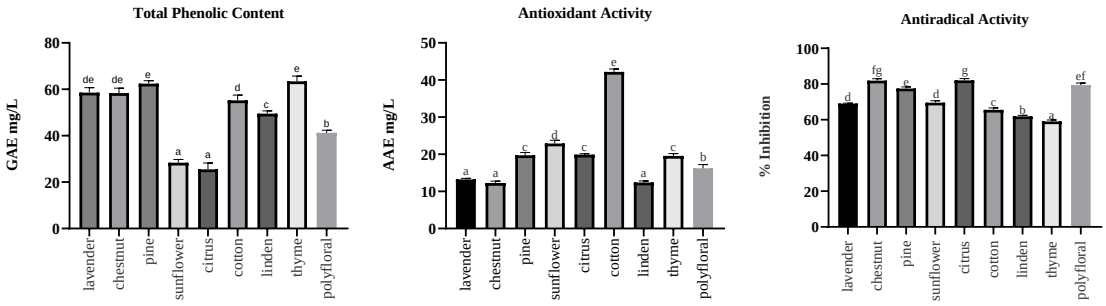

Figure 1- TPC, antioxidant and antiradical activity of honeys

The inhibition values of honey samples are between 58-92%. The honeys with the highest inhibition value (radical scavenging activity) are chestnut and citrus honeys. Antiradical activity from high to low is as follows; citrus, chestnut, polyfloral, pine, sunflower, lavender, cotton, linden, and thyme.

4. Discussion and Conclusion

The botanical origin of a honey is based on the relative frequencies of pollen types represented by nectariferous species in the honey. If pollen from a plant species exceeds 45% in the honey, that honey can be defined as unifloral/monofloral. In our study, citrus honey fell below this rule. However, honey does not sufficiently represent certain pollen types (*Citrus* spp., *Tilia* spp.). For Citrus honey to be classified as monofloral, it must contain at least 10% Citrus spp. pollen (Oddo et al. 1995).

In the honey samples tested in this study, the lowest moisture content was detected in linden and polyfloral honeys, followed by cotton, thyme, pine, lavender, chestnut, sunflower, and citrus honeys, respectively. Citrus honey in this case exhibited the highest moisture content. Yıldız et al. (2008) found that the moisture percentage in the honeys (pine, cotton, polyfloral, and sunflower honeys) was higher than the values determined in our research. Can et al. (2015) determined the moisture content of nine monofloral honeys to be between 16.54 and 20.86%. The moisture content of honey varies depending on floral sources, beekeeping activities, and climatic conditions. Generally, the water content of honey is less than 18%. The Codex Alimentarius and the European Directive set limits for moisture content of not more than 20%. The sugars found in honey at the highest levels are glucose and fructose. In our study, sunflower honey had the highest fructose content, while pine honey had the lowest fructose content. In all of the monofloral honeys tested in this study, fructose was dominant, i.e., it was more abundant than all other sugars, including glucose. Escuredo et al. (2014) found the glucose content of rapeseed honey to be higher than fructose in their study. Moreover, acacia honey exhibited the highest fructose concentration (42.3%), surpassing the values of thistle, chestnut, eucalyptus, and honeydew honeys. They discovered that honeydew honey, also known as pine honey, had a relatively low fructose content of 32.30%, which aligns with our research findings. In our research, we found that citrus honey had the highest glucose content, while thyme honey had the lowest. In the research conducted by Escuredo et al. (2014) it was found that sunflower and rapeseed honeys had significantly higher glucose contents than other honeys, and the lowest glucose content was detected in honeydew honey. In fact, it is known that honeys with a glucose content below 30% exhibit slow crystallisation (Manikis & Thrasivoulou 2001). Honeydew honey, chestnut, heather, and acacia honeys exhibit show crystallisation, whereas sunflower, linden, and rapeseed honeys show fast crystallisation (Escuredo et al. 2014). This study determined the sucrose content of lavender, chestnut, pine, sunflower, citrus, cotton, polyfloral, linden, and thyme honeys tested were 1.2, 0.2, 1.9, 1.4, 1.9, 2.2, 1.2, 1.4, and 2.2%, respectively. Another study determined that acacia honey had the highest sucrose content (2.3), while chestnut and eucalyptus honeys (0.2) had the lowest sucrose content (Escuredo et al. 2014). Oddo & Piro (2004) attributed the rapid crystallisation of sunflower honey to its high glucose content, and the long-term non-crystallisation of chestnut honey to its high fructose and low glucose content. Honeybees prefer chestnut, eucalyptus, and honeydew honeys as their primary nectar sources. While sucrose, maltose, trehalose, and turanose are the main oligosaccharides and disaccharides in flower honeys, oligosaccharides such as hybridose, erlose, and raffinose are found in higher amounts in secretion honeys (Földhazi 1994). Moroccan, Romanian, Croatian, and Macedonian honeys have high amounts of hybridose, one of the important marker compounds of honeydew honey (Terrab et al. 2003; Primorac et al. 2009; Dobre et al. 2012).

HMF is used as an indicator of honey freshness. It is usually absent or present in very low amounts in fresh honey. High *HMF* values could also suggest that inverts syrups have contaminated the honey (Swallow et al. 1994; Terrab et al. 2002). Criteria have been established to determine the limits of HMF content in honey. The Codex Alimentarius (2000) specifies that the *HMF* content should not exceed 80 mg kg^{-1}, while the Council Directive 2001/110/EC of the European Community (EU) (2002) sets the *HMF* limit in honey at 40 mg kg^{-1}, with some exceptions. All honeys in this study had HMF values below 40 mg kg^{-1}. Our research determined the HMF content of honey samples to range from 1.8-22.3 mg kg^{-1}, the lowest value was found in sunflower and the highest *HMF* value was found in thyme honey. Another study (Can et al. 2015) determined the *HMF* content of 11 monofloral honeys produced in our country to range between 0.61-62.24 mg kg^{-1}. Unlike this study, no honey above the limits specified in the honey circular (<40 mg kg^{-1}) was detected in our research. Various parameters were used in the evaluation of the freshness of honey (Bogdanov 1997; Mendes et al. 1998; Küçük et al. 2007). The honeybee secretes diastase, one of these parameters, which aids in starch digestion. When we compared the enzyme activities of the honeys tested in our research, we found that diastase activity is between 3.6-9.2 (*DN*) and the lowest is citrus and the highest is chestnut honey, while invertase activity is between 26.8-265.6 U kg^{-1}, and the lowest is thyme and the highest is chestnut honey. Can et al. (2015) found that the diastase content of our country's honeys ranged from 6.30 to 12.60 *DN*, whereas it was seen that pine and chestnut honeys have higher diastase activity in our research. We found that the diastase activity of the tested honeys, except citrus and pine honey, exceeded the legal limit of 8 DN (EU, 2002). This result can be explained by poor processing of nectar by bees, bee-related factors, beekeeping activities, and environmental conditions.

Proline, the essential amino acid in honey, is both a criterion for its maturity and also serves to determine adulteration. Proline levels of adulterated honey are low (Can et al. 2015). High proline content is typical for some types of monofloral honey (Ouchemoukh et al. 2007). According to the honey Codexes (Turkish Food Codex and Codex Alimentarius), the desired proline level in honey is 250 mg kg^{-1}, and this level is much higher in quality honeys (Al-Mamary et al. 2002). We determined the lowest proline values of the honey samples we tested to be 290.8 mg kg^{-1} in citrus honey. Another study found that proline levels range from 282 to 845 mg kg^{-1}, with chestnut and heather honeys exhibiting higher proline (Can et al. 2015). The acidity of honey is a feature responsible for its taste, aroma, and antibacterial activity. In our study, the acidity values of the honey samples varied between 11.6-27.0, lowest acidity value was observed in citrus honey, and the highest acidity was observed in thyme honey.

We used bioactivity tests to quantitatively measure of honeys biological activity. Honey contains many bioactive components, such as phenolic compounds, enzymes, and organic acids, which contribute to the biological and therapeutic effects of honey (Abou-Shaara et al. 2015). Among these compounds, phenolic compounds are some of the most important compounds contributing to the antioxidant activity of honey. TPC is also an indicator of the antioxidant capacity of honey (Can et al. 2015). In this study, a statistically significant difference was determined between the *TPC*, antioxidant, and antiradical activities of the honey samples tested ($P<0.05$). *TPC* of the honeys analysed in our study varied between 49.5-63.42 mgGAE/100g. Thyme honey had the highest phenolic content, while linden honey had the lowest. The antioxidant activity of the honeys varied between 12.41-42.20 µg/mL, and the antiradical activity varied between 59.04-81.90%. Upon comparing the antioxidant activity of honey samples, cotton honey demonstrated a higher antioxidant activity than other honeys. Chestnut honey showed higher antiradical activity than other honeys. The reasons for the different results obtained in studies examining the antioxidant activity of honeys were attributed to the different geographical and botanical origins of honeys and environmental and climatic differences (Buratti et al. 2009). Some studies have shown that dark coloured honeys contain higher amounts of phenolic substances and have higher antioxidant activity (Küçük et al. 2007; Haroun 2006). For example, it has been reported that chestnut honey, which is a dark coloured honey, has high TPC, and pine honey has high antioxidant activity (Akbulut et al. 2009; Silici et al. 2010; Özkök &Silici 2017).

As a result, consumers generally prefer honey according to its sensory properties. It is clear that monofloral and polyfloral honeys show different physicochemical and bioactivity. However, the quality of honey is the most important reason for consumers to prefer it. This study determined that all honeys, except for diastase activity, were within the Codex limits.

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