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Characterization of Turkish pine honey according to their geographical origin based on physicochemical parameters and chemometrics

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Abstract

This study was conducted to determine the characteristic properties of Turkish pine honey, which is an important honeydew honey. The geographical classification of the honey was determined by applying carbon isotope, melissopalynological, and physicochemical analyses to 373 samples collected from 47 regions between 2015 and 2017 under controlled conditions. δ^{13}C protein-δ^{13}Choney, C4%, electrical conductivity, moisture, ash, free acidity, color CIEL* a*b attributes, optical rotation $[\alpha]^{20}$, proline, diastases activities, and sugars (fructose, glucose, sucrose, and maltose) were used as physicochemical properties. Number of honeydew elements /number of total pollen (NHE/NTP) ratios were studied at melissopalynological analyses. The results showed that all samples exhibited honeydew properties, and that all physicochemical parameters met the criteria set by regulatory standards for honeydew. However, C4% sugar and δ^{13}C protein-δ^{13}C honey values did not meet the regulatory criteria and exhibited quite wide standard deviations.

Keywords Pine honey · Honeydew · Turkish · Physicochemical · C4 sugar

Introduction

Honey is a natural food produced by honey bees (*Apis mellifera* L.) from two different sources. One form is blossom honey produced from flower nectars, such as acacia, chestnut, lavender, and heather. The other form is known as dew or secretion honey, also produced in two different ways. One method involves collection from various sugars or liquids leaking from trees, leaves, and fruits, such as oak (*Quercus frainetto*), spruce, and fir, in the form of sweating, depending on climatic conditions [1–3]. Another form involves the collection of excretions of sucking insects, such as aphids, white flies, and mealy bugs, or soft scale insects such as *Marchalina hellenica* (syn. *Monophlebus hellenicus*), *Lachnus iliciphilus* and *Thelaxes dryophila*., (Coccoidea: Homoptera) is mostly found in the Aegean and Mediterranean regions and lives on pine tree species such as *Pinus brutia* and *Pinus pinea* [4]. Most of the world's pine honey (nearly 90%) is produced on the Aegean and Mediterranean coasts of Turkey, since the climatic conditions and relative humidity are well suited to *M. hellenica*. This honey is also produced in smaller quantities in Greece, Italy, Portugal, and

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Spain [5]. In Turkey, most pine honey production is carried out in or around the province of Muğla. Some 10,000 bee-keepers produce 40,000 tons of pine honey annually in these regions. Due to its low pollen and high fructose levels, the honey does not crystallize easily. It therefore always exhibits the characteristics of raw honey and requires no thermal treatment [6]. This honey is also relatively dark in color and contains high levels of phenolic compounds and as well as exhibiting significant antioxidant, anti-inflammatory, and antimicrobial properties [7].

Pine honey exhibits different physicochemical properties in terms of production and composition to other blossom and honeydew honeys. This honey is produced during two or three harvesting times ranging from September to December. The first honey produced may be mixed with various flower honeys, although pure honeydew honey is obtained at other times. Since the composition of this honey varies depending on the collection time and climatic conditions, Turkish pine honey manufacturer sometimes suffers from peculiarity problems in C4 sugar content exceeding the acceptable level of 7% [8, 9]. However, honey bees mostly collect nectar from flowers of C3 plants, and to a lesser extent from the flowers of C4 and CAM plants, with different $^{13}C/^{12}C$ ratios [10, 11].

It is unclear whether this variation is due to the producer or to geographical conditions. Although some blossom and honeydew honey properties have been determined in the Turkish food codex, the characteristic features of Turkish pine honeys are not yet fully described. This study was performed to identify the specific properties of Turkish pine honey. This research was planned as a comprehensive study under controlled conditions, and differences in place and time were not taken into consideration.

Materials and methods

Collection of samples

This study was conducted by the Turkish Beekeepers Association and supported by the Turkish Ministry of Agriculture and Forestry, to determine Turkish pine honey criteria. The honey samples were collected from three geographical region of Turkiye, mostly from the Aegean coast, with some from the Marmara and Mediterranean shores. Three hundred seventy-three honey samples were collected from 47 regions from September to December in 2015 ($n=61$), 2016 ($n=175$), and 2017 ($n=137$) years (Fig. 1). All honeys were produced under controlled conditions with no sugar-feeding or adulteration.

Marchalina hellenica areas of each location were taken into consideration in the selection of the location of the colonies. Honey samples were produced for 3 years in seven different provinces under the project (Fig. 1). All samples were collected in triplicate and stored under cool conditions.

Melissopalynological analyses

Pollen analysis is performed microscopically and ratio of number of honeydew elements (NHE) to number of total pollen (NTP) was determined according to [12, 13] and [14] methods. The microscopy of pine honey differs in some respects from that of blossom honeys. Honeydew honeys contain lower pollen numbers, whereas more fungi spores and hyphaes. Therefore, in this study, total pollen and honeydew element numbers in honey samples were examined under the light microscope (Nikon Eclipse E400, Germany).

Honeydew honey is generally characterizedby honeydew elements composed of microscopic algae, fungus spores. If a honey with the ratio "number of honeydew elements (NHE)"/"number of total pollen (NTP)" is greater than 3, it is considered as honeydew honey (Louveaux et al., 1978; Soria et al., 2004). Microscopic preparations prepared were prepared using Lycopodium spores with the classic melissopalynological assay for determination of NHE and NTP [13, 15]. The calculations were performed using the two-formula given below in 10 g honey:

Number of total pollen (NTP)/10 g = Counted pollen number × 12,542*/Counted Lycopodium Spores.

Number of honeydew elements (NHE)/10 g = Counted honeydew elements number × 12,542*/Counted Lycopodium Spores.

*= Number of spores that is found in a one Lycopodium spore tablet.

Carbon isotope values and C4 sugar analyses

The official [17] method was employed to determine the carbon isotopes ($\delta^{13}C$) in the honey samples (Anonymous, 2006). The principle of this analytical method is based on the determination of the $^{13}C / ^{12}C$ ratio ($\delta^{13}C$) of the C atom in CO_2 from the combustion of raw honey and precipitated protein using elemental analyzer-isotope spectrometry (EA-IRMS) (Thermo Fisher P2000 Elemental Analyzer-Isotope Ratio Mass Spectrometry). The C4% sugar is calculated by the [8] standard according to the following equation [18]:

$$C4\% \ sugar = [\delta^{13}Cprotein - \delta^{13}Choney] \times 100 \ / \ \delta^{13}Cprotein-(-9.7).$$

Physicochemical analyses

The moisture, free acidity, ash content, viscosity, and electrical conductivity of honeys were measured using the harmonized methods of the International Honey Commission [19]. Sample optic rotation was measured in a polarimeter (Kruss P 3000, Germany) using Carrez's reagent precipitation of
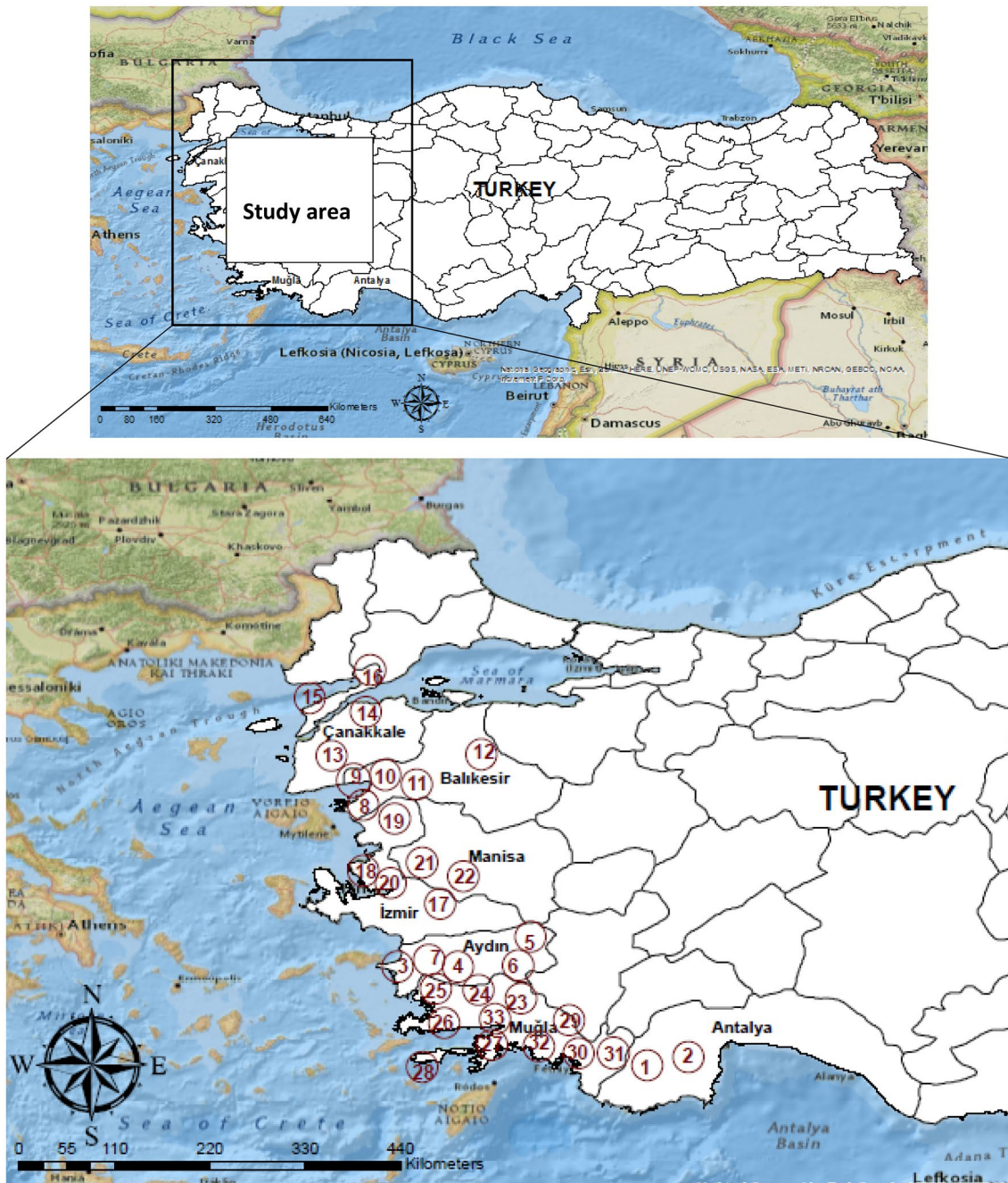
Fig. 1 The regions where the pine honeys were produced

honey proteins. The filtrate solution was inserted into the polarimeter, and the results were stated in angular on a 200 mmol basis [20].

Color characteristics of the samples were measured using Hunter Lab tritium color (Konica, Minolta, and CM-5, Minolta, Osaka, Japan). The apparent colors of the honeys were measured in terms of (L) for darkness/lightness (0 black, 100 white), a (−a greenness, +a redness), and b (−b blueness, +b yellowness [21].

Viscosity was measured using a viscometer (DV-II Viscometer No. MO3-165-E = 211) (Brookfield, UK). The device has a measurement range of 1000- E = 211) (Brookfield, UK). The device has a measurement range of 1000—a range of 40–60% at 20–25 °C.

The spectrophotometric method was used for quantitative determination of the proline content [19]. This method is based on the color reaction of proline with ninhydrin

reagent, and absorbance was read at 510 nm. The results were expressed in mg/kg.

The diastase activity of the samples was measured using a photometric method in agreement with the IHC [19], in which an insoluble blue dyed cross-linked type of starch is used as the substrate. This is hydrolyzed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm. The results are expressed in Gothe units (or Schade units) per gram of honey.

Sugar profiles were determined by injecting samples into vials under the same device conditions [22]. Sugar fractions of the honey samples were measured with chromatographic methods using a refractive index detector (HPLC-RID) (Shimatzu 10 A, Japan). HPLC analyses of the sugar profiles were carried out on Shodex NH2P-50 4 E column (5 µm, 250×4.6 mm). Chromatographic separation was achieved using isocratic elution with acetonitrile/water (75:25, v/v) at a flow rate of 1.0 ml/min. The column was maintained at 30 °C. Pure glucose, fructose, sucrose, and maltose standards were first prepared with 0.15%, 0.25%, 0.50%, and 1% ultra-pure water, filtered through a Millipore 0.45 µm PVDF filter, and given to the device, when a four-point calibration curve was drawn. For preparation of the honey sample, 1 g sample was weighed into a flask, and 50 ml of ultra-pure water was then added. The mixture was homogenized by vortexing using a pipette to a volume of ultra-pure water.

Statistical analyses

The samples were subjected to the Kolmogorov–Smirnov test to assess normality for the statistical analyses. The one-way Analysis of Variance (ANOVA) was used for comparison of $\delta13C$ protein, and $\delta13C$ protein-$\delta13$ C honey means by years. On the other hand, Kruskal–Wallis test was used to assess the year differences for $\delta13C$ honey and C4 sugar, also physicochemical and Palynological analysis means. Accordingly, while mean and standard errors are presented in ANOVA results, mean values for Kruskal Wallis are presented with standard deviation values. In addition, the analysis used in the change of the mean of the parameter values over the years is shown as a superscript in the row of the relevant tables. Duncan test was used for multiple comparison of means obtained by ANOVA, and Dunn's Bonferroni test was used for multiple comparison of mean rank according to Kruskal–Wallis test.

Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) were used for chemometric analysis of pine honey samples. Cluster analysis was employed to get hierarchical relations using the Ward Linkage method and Euclidean distance. Thus, PCA results in the study were also supported by hierarchical cluster analysis (HCA). All statistical analysis were performed using IBM SPSS v25 and MINITAB 16.0 softwares.

Results and discussion

Melissopalynological analyses

Since pine honey is a honeydew product, it always contains lower pollen and higher honeydew elements than blossom honeys. NTP, NHE, and the ratio of NHE/NTP are considered an important criterion in the determination of honeydew honeys.

An NHE/NTP ratio of 0–1.5 has been defined as representing blossom honey, a ratio of 1.5–3.0 as mixed blossom and honeydew honey, and higher than 3.0 as honeydew honey [13, 14]. The descriptive statistics of Palynological analysis of Turkish pine honey and comparison results by years are given in Table 1. The NHE/NTP ratios in the present research are varied between 0.05 and 360, with a mean value of 24.21 ± 41.86. The results show that all the honeys in this study were honeydew elements, but that the honey samples collected in 2015 had better quality honeydew honey properties. There may be several reasons for the variations in the NHE/NTP ratio in the different years, including climatic changes in particular, the status of the bee colonies, and changes in honeydew elements levels in pine trees with a decrease in *Marchallina hellenica* numbers. However, the NHE/NTP ratio is not by itself sufficient for determining the characterization of pine honeys, and it should be evaluated together with electrical conductivity and optical conversion parameters. The NHE/NTP ratio varied between 0.02 and 106 in 78 pine honeys from 2010 in one study, half being high-density honeydew honeys [14].

Table 1 Descriptive statistics of palynological analysis for Turkish pine honey and comparison of means by years

Palynological analyses	Min–Max	Mean ± SD	2015 ($n=61$)	2016 ($n=175$)	2017 ($n=137$)	P values
NHE/NTP	0.05–320.00	24.21 ± 41.86	33.48 ± 5.63^a	18.84 ± 4.09^b	20.31 ± 3.17^{bc}	< 0.05 [2]

Different superscript letters represent significantly differences ($p < 0.05$)

[2] Kruskal–Wallis test was applied and the mean ± standard deviation was defined

Carbon isotope analyses

Carbon isotope parameters of δ^{13}Cprotein (‰), δ^{13}C honey (‰), δ^{13}C protein- δ^{13}C honey, and C4% sugar in the pine samples are given in Table 2. The values are expressed as mean and standard deviation in table. The descriptive statistics of the carbon isotope values of the Turkish pine honey and their differences according to the 3-year study period are summarized in Table 2 with mean, standard deviation, and standard errors. The results revealed significant differences in C4% sugar levels by years. The values ranging from significant differences in C4% sugar levels by years and the values ranging from 3.45 ± 3.74 to 7.67 ± 5.07. The lowest C4% was determined in 2015 and the highest in 2016. The carbon isotope and C4% sugar values of 373 honey samples during the 3 years are summarized in Table 2. δ^{13}C protein values of the honeys ranged from -27.19 to -21.97, with a mean value of -24.59 ± 0.87.

δ^{13}Choney values ranged from -27.5 to -21.71, with a mean value of -23.75 ± 1.17. The difference between δ^{13}C protein and δ^{13}C honey is ranged from -3.63 to 5.79. The amount of C4% sugar calculated according to the δ^{13}Choney and δ^{13}Cprotein isotope values varied between 0 and 22, with a mean value of 6.40 ± 5.00. Although the mean C4% values for all the honey samples were below the acceptable value of 7.0 specified under the IHC regulation. [8], the standard deviation was very large. Most of the honey samples had C4% values above 7.0. The difference in C4% sugar values during the study was an expected finding, although no sugar was fed during the harvesting time. The locations where the samples were collected were not considered in the present study, only the differences between the years being examined. C isotope analysis in honey is mostly used for adulteration tests. However, the accuracy of these tests is controversial. When C3 plants are used in the production of any honey species, the C4% sugar content of the honey must be below 7% [23]. While C3 type sugars are produced from sugar beet, apple, and grapes, C4 type sugars are produced cane and corn, In the honey codex, ^{13}C / ^{12}C values between

-22% and -33% are regarded as acceptable in C3 plants, -10% to -20% in C4 plants, and -11.0% to -13.5% in CAM plants [10]. These differences can be used to show whether foods from different photosynthesis groups have been mixed [10]. Adulteration in honey is a very important subject and is performed in different ways. High C4 sugar content is reported in honey obtained from bees fed with cheap sugars, such as sugar cane and corn syrup; although sugar cane is not used in Turkey, beet sugars such as sucrose generally being used instead [10]. It is thought that the high C4% sugar values found may be the result of geographical features, but not any adulteration. Similar results have also been reported in Manuka honeys, which have also been found to be incompatible with the AOCC specifications [11]. In one study of Turkish pine honey, δ^{13}C protein values were reported to exhibit significant differences over the years [24]. Another study reported no change in δ^{13}C protein and δ^{13}C honey and C4% sugars in adulterations with sugar syrups of controlled feeding [9]. In its current form, carbon isotope analysis findings should not be used as adulteration parameters in pine honey. Amendments for pine honey are therefore required in domestic and international legislation.

Physicochemical analyses

The physicochemical analysis results for the pine honey in the different study years are summarized in Table 3. The descriptive of statistical values of the physicochemical analysis results of pine honey in different study years are summarized in Table 3. Moreover, the differences in the physicochemical properties of 373 pine honey samples collected from 47 regions between 2015 and 2017 are given in Table 4.

The mean moisture percentage of the honey samples by years varied between 15.01% and 17.66%. However, in terms of the percentage of moisture for samples collected over the 3-year study period, the minimum moisture was 13% and the maximum was 21.90%. According to international standards, while there may be exceptions in moisture levels,

Table 2 Descriptive statistics of carbon isotope values of Turkish pine honey and comparison of means by years

	Min/Max	Mean ± SD	2015 n:61	2016 n:175	2017 n:137	*P* values
δ^{13}C protein (‰)	$-27.19/-21.97$	-24.59 ± 0.87	-25.71 ± 0.05^a	-24.46 ± 0.03^b	-24.29 ± 0.04^c	<0.01 [1]
δ^{13}C honey (‰)	$-27.50/-21.71$	-23.75 ± 1.17	-25.38 ± 1.29^a	-23.38 ± 0.68^b	-23.50 ± 1.01^b	<0.01 [2]
δ^{13}C protein-δ^{13} Choney	$-3.63/-5.79$	-0.83 ± 1.01	-0.34 ± 0.11^a	-1.04 ± 0.08^b	-0.79 ± 0.08^b	<0.01 [1]
C4 sugar (%)	$0.00/-22.25$	6.40 ± 5.00	3.45 ± 3.74^c	7.67 ± 5.07^a	6.11 ± 4.85^b	<0.01 [2]
Number of C4 samples > 7%	–	–	10	87	48	–

Different superscript letters represent significantly differences ($p < 0.01$)

[1] One-way ANOVA was applied and the mean ± standard error was defined

[2] Kruskal–Wallis test was applied and the mean ± standard deviation was defined

Table 3 Descriptive statistics of physicochemical analysis results of 357 pine honey samples

	Minimum	Maximum	Mean ± SD
Moisture (%)	13.00	21.90	16.07 ± 1.73
Optical rotation $[\alpha]^{20}$	0.08	2.39	1.06 ± 0.53
Color CIEL* a*b			
Hunter L (0 black, 100 white)	18.96	75.70	58.62 ± 9.46
Hunter a (− greenness, + redness)	7.39	40.14	19.58 ± 4.90
Hunter b (− blueness, + yellowness)	24.58	98.71	76.02 ± 8.61
Conductivity (mS/cm)	0.73	1.94	1.37 ± 0.23
Ash (%)	0.21	1.85	0.70 ± 0.26
Viscosity (23 °C)(Pa.s)	0.25	16.20	2.74 ± 1.82
Total acidity (meq/kg)	8.00	46.89	18.57 ± 5.62
Proline (mg/kg)	158	961	408.57 ± 138.38
Diastase Unit (DU)	4.10	32.36	14.11 ± 4.83
Fructose (%)	19.54	44.72	28.73 ± 4.18
Glucose (%)	16.29	41.52	25.55 ± 3.79
Sucrose (%)	nd	7.87	1.84 ± 1.85
Maltose (%)	nd	nd	nd
Fructose/glucose	0.70	1.51	1.13 ± 0.09
Glucose + fructose (%)	36.62	86.15	54.28 ± 7.68

nd Not detected

Table 4 Comparison of physicochemical analysis means by years

	2015 n=61	2016 n=175	2017 n=137	P values
Moisture (%)	17.66 ± 1.41[a]	15.01 ± 0.91[c]	16.72 ± 1.79[b]	<0.01 [2]
Optical rotation $[\alpha]^{20}$	0.75 ± 0.47[b]	0.88 ± 0.44[b]	1.42 ± 0.44[a]	<0.01 [2]
Color CIEL* a*b Hunter L (0 black, 100 white)	78.47 ± 6.60[a]	74.97 ± 6.11[b]	76.26 ± 11.49[a]	<0.01 [2]
Hunter a ((− greenness, + redness)	19.07 ± 4.91[b]	18.63 ± 4.00[b]	21.02 ± 5.57[a]	<0.01 [2]
Hunter b ((− blueness, + yellowness)	78.47 ± 6.60[a]	74.97 ± 6.11[b]	76.26 ± 11.49[a]	<0.01 [2]
Conductivity (mS/cm)	1.51 ± 0.11[a]	1.34 ± 0.22[b]	1.35 ± 0.25[b]	<0.01 [2]
Ash (%)	0.66 ± 0.10[b]	0.65 ± 0.22[b]	0.78 ± 0.33[a]	<0.01 [2]
Viscosity (23 °C)(mPa.s)	1.58 ± 1.58[c]	3.50 ± 1.45[a]	2.30 ± 1.92[b]	<0.01 [2]
Total acidity (meq/kg)	23.16 ± 7.43[a]	17.60 ± 4.45[b]	17.78 ± 5.01[b]	<0.01 [2]
Proline (mg/kg)	395.50 ± 119.55[ab]	399.06 ± 138.47[b]	426.54 ± 144.55[a]	0.03 [2]
Diastase unit (DU)	17.35 ± 5.34[a]	13.52 ± 4.63[b]	13.44 ± 4.24[b]	<0.01 [2]
Sucrose (%)	0.26 ± 0.63[a]	0.90 ± 1.14[b]	3.73 ± 1.27[c]	<0.01 [2]
Maltose (%)	nd	nd	nd	nd
Glucose (%)	25.85 ± 4.51[a]	23.72 ± 2.76[b]	27.74 ± 3.36[c]	<0.01 [2]
Fructose (%)	28.80 ± 4.04[a]	26.14 ± 2.98[b]	32.00 ± 3.12[c]	<0.01 [2]
Glucose + fructose (F + G)(%)	54.68 ± 8.30[a]	49.86 ± 5.44[b]	59.73 ± 6.14[c]	<0.01 [2]
Fructose/glucose (F/G)	1.13 ± 0.10[a]	1.11 ± 0.08[a]	1.16 ± 0.09[b]	<0.01 [2]

Different superscript letters represent significantly differences ($p<0.01$), nd: not detected

[2] Kruskal–Wallis test was applied and the mean ± standard deviation was defined

the amount should not exceed 20%. It has been observed that the amount of moisture varies depending on the geographical features, harvest time, and terrain structure of the region where the honey is collected (Tables 3 and 4). The water content of honey varies depending on the harvest period, climatic factors, and the degree of maturity reached in the hive [25]. The honey samples with the mean lowest and highest moisture contents were from the 2016 harvest year and 2017, respectively. The Honey Codex states that the maximum moisture in honey should not exceed 21%,

beyond which fermentation may easily occur. Harvesting honey before maturation can result in high moisture levels [26, 27].

The optical rotation values of the honey samples are also given in Tables 3 and 4. All the measured optical rotation values were positive, ranging from 0.80 to 2.39 in the pine honey samples. The mean optical rotation value for the 3 years was 1.06 ± 0.53. The highest average optical rotation value was observed in 2017. Optical rotation values are another important physical parameter for sugars, and result from the fact that some sugars turn polarized light to the right and others to the left. The optical rotation value is always positive in honeydew honey and negative in blossom honey [7, 20]. The optical rotation value distinguishes between honeydew and blossom honey better than the pollen count.

Similar to the current research, the previous studies have also reported that optical rotation values are always negative in blossom honeys and always positive in dew honey [1, 26]. Furthermore, the compatibility between the honeys' NHE/NTP values and the optical rotation values indicates that they possess the characteristics of secretion honey. Another of the important characteristics of the Turkish pine honey is its color. The color of the honeys in the present study was determined based on the Hunter L values, as summarized in Tables 3 and 4. The Hunter L values, indicating the darkness and lightness of honey, varied between 18.96 and 75.70, and the mean value for the 3 years were calculated as 58.62. No marked differences were observed among the 3 years. The darkest Hunter L value was found 2016(74.97 ± 6.11). A study from Greece investigating pine honeysreported L* values between 66 and 75 [5]. A study involving a small number of pine honeys from Turkey determined a mean Hunter L value of 54.38 ± 4.39 [7]. Many factors, such as the secondary metabolites (polyphenols, anthocyanins), vitamins (A, E), pigments, pollens, minerals, and Maillard reaction products, are involved in determining honey colors (28, 7, 29, 4). There is also a relationship between the honey color and the amount of 5-hydroxymethylfurfural (HMF) contained.

HMF is the criterion determining the freshness of honey and has been shown to increase in line with shelf life and exposure to light. However, the rate at which HMF develops varies depending on the type of honey (lightness, darkness, etc.) [28]. The second honey color, Hunter a*, shows the greenness and redness of a foodstuff. Hunter a value in the present study ranged from 7.39 to 40.14, and the mean value over the 3-year study period being calculated as 19.58 ± 4.90 (Table 3). All the Hunter values were below 40 in the honey samples, and the results indicated that Turkish pine honeys are almost greenish in color. Little change was observed when the average Hunter a*values were compared between the years, values ranging 18 and 21. One study reported

negative Hunter a value for Mediterranean region honeys with a multifloral character, ranging from -3.64 to -6.27 [30]. Another study of oak honey (*Quercus* spp.) reported that Hunter a* values varied between 16 and 42 [1]. A mean Hunter a value of 26.80 ± 2.63 has also been reported in a small number of Turkish pine honeys (7, 28).

The third Hunter color value is b*, which indicates blueness and yellowness. The lowest Hunter b* value in the 373 pine honeys in the present study was 24.58 and the highest Hunter b* value was 98.71, with a mean value of 76.02 ± 8.61. The mean values in the different years varied between 74.97 ± 6.11 and 78.47 ± 6.60. These results shows that Turkish pine honeys have a more yellowish character. A study conducted on monofloral Turkish honeys reported Hunter b values of 5 in clover honey, 81 in chestnut honey, 32 in acacia honey, and 89 in pine honey [7]. A study from Greece reported a Hunter b value for pine honey of 31.07 [18]. Analysis of the color parameters of the honeys in the present study revealed that Turkish pine honey was greenish and yellowish in color, with medium darkness. Although there is no provision regarding the color parameter in the Turkish Food Codex on Honey Legislation, the results of the color analysis were compatible with the ranges previously reported in the literature [28, 31]. The difference in Hunter a and b values in pine honeys measured between the two neighboring countries of Turkey and Greece is due to differences in plant flora.

Another rheological property measured in pine honeys is conductivity. The electrical conductivity values of the honey samples in the present study are given in Tables 3 and 4. The lowest conductivity detected in the honeys was 0.73mS/cm and the highest 1.94 mS/cm, with a mean value of 1.37 ± 0.23 mS/cm. The conductivity of honey varies according to its botanical origins and the amounts of Na, K, minerals, acidity, organic acids, and polyphenols it contains. The minimum and maximum limits of light color blossom honey set by the EU [38] are 0.30mS/cm and 0.80 mS/cm, although conductivity differs in dew and some dark color honeys, such as chestnut, oak, fir, and pine. The electrical conductivity of honeydew honey and dark colored forest honeys is usually higher than 0.80 mS/cm [30, 32].

The residue that occurs after honey is burned at high temperatures is known as ash, an important indicator of its mineral content. The ash values measured in pine honeys in this study varied between 0.21% and 1.85, with a mean value of 0.70 ± 0.26. Total ash contents of 0.30–0.79 have been reported in dew honey [1] and 0.16% and 0.22% in blossom honey [33].

Similar to moisture, viscosity is another important honey parameter. The viscosity of honey is an essential rheological property and varies depending on water content, brix value, temperature, and crystallization state. Honeys with high viscosity are generally preferred by consumers, and viscosity is

reported to vary in line with water content and temperature [34, 35]. A mean viscosity value of 2.74 ± 1.82 mPa.s was determined in the 373 pine honeys collected over a period of three years (Table 3), ranging from 0.75 to 16.20 mPa.s (Table 4). The mean viscosity values varied between 1.58 and 3.50 mPa.s depending on tree harvest years (Table 3). Viscosity values differed significantly between the years. Pine honey viscosity was highest in 2016, and the lowest amount of moisture was also observed in that year. A study of Ethiopian monofloral honey reported viscosity values at 25 °C of 6.14 and 29.21 mPa.s [36]. Another study of monofloral Turkish honeys reported viscosity values between 1.77 and 11.20 mPa.s. The viscosity value of pine honey in that study was 8.44 [37].

Total or free acidity values were measured in the pine honey samples as a physicochemical and rheological parameter and are given in Tables 3 and 4. Total acidity values varied from 8.0 to 46.89 meq/kg, with a mean value of 18.57 ± 5.62 meq/kg. The total acidity value of the honey samples was highest in 2015, and there were no significant differences in 2016 or 2017. A maximum total acidity value of 50 meq/kg is recommended in the honey codex [23, 38]. Our findings were thus compatible with the existing legislation. The acidity of honey derives from various ions and salts and numerous different organic acids, phenolic acids, and low amounts of fatty acids [39].

Fermentation also increases the acidity of honeys [40, 41]. Free acidity of 18.08–41.54 meq/kg was reported for pine honey by [4] and 4–43.3 mg/kg for honeydew by [42].

An important parameter responsible for the freshness and quality of honey is enzymatic activity. Although diastase, invertase, and glucosidase are major enzymes in honey, diastase activity is routinely measured in honey to determine freeness and heating processes, as well as raw honey according to the IHC, Codex Alimentarius Honey Standard, and the European Union Honey Directive, this situation is not the same for raw honey [43]. The diastase activities of the honey samples in the present study are summarized in Tables 3 and 4. Diastase activities varied between 4.10 and 32.36 units, with a mean value of 14.11 ± 4.83 units. The lowest average diastase unit was measured in 2017 (13.44 ± 4.24) and the highest value in 2015 (17.35 ± 5.34). European legislation [38] imposes a minimum value of 8 on the Schade scale for diastase activity. This confirms that the diastase activities of the honey are compatible with the Honey Codex. Diastase activity varying from 10.90 to 13.90 was reported in one study of pine honey [37].

Proline is the major amino acid in honey, although all amino acids are present in trace amounts. The amount of free proline amino acids in honey is used as an important marker in distinguishing genuine honey from fake honey. However, proline values are reported to decrease significantly in honey fed with sugar syrup [12]. The minimum amount of proline required in honey varies depending on individual countries' honey codices. While the minimum proline value is 180 mg/kg under IHC and European regulations, the Turkish Honey Codex accepts 300 mg/kg [19, 23, 38]. The proline values of the pine honeys in the present study varied from 158 to 960 mg/kg, with a mean value of 408.57 ± 138.38 Table 3. No major difference was also determined in mean values between the years. Another study of Turkish pine honeys reported proline values of 301–977 mg/kg [44], similar to the range of proline content reported in Greek pine and fir dew honeys [30].

The dry matter of honey consists of 98% carbohydrates. Fructose and glucose are the major monosaccharides of honey, together with lower levels of sugars, such as sucrose, maltose, melezitose, melebiose, turanose, etc. As with other components, the sugar composition of honey also varies according to the plant flora [42]. HPLC-RID analysis revealed mean fructose percentages between 28.80 ± 4.04 and 32.00 ± 3.12 in the different years, while glucose varied between 25.85 ± 4.51 and 27.74 ± 3.36 [Table 4]. Similarly, the sucrose percentage ranged from 0.26 ± 0.63 to 3.73 ± 1.27, while no maltose was detected.

Significant differences ($p < 0.01$) were determined between fructose and glucose values by years. Fructose/glucose ratio values ranged between 0.70 and 1.51, and fructose + glucose percentages ranged between 36.62 and 86.15 for all years Table 4. These results were in accordance with the Turkish Food Codex Notification on Honey [23] and the IHC regulation [38]. The fructose/glucose is an important factor in the crystallization of honey; the higher this value is 1.0, the later the honey crystallizes [29]. The average sucrose values in the honey samples ranged between 0.26% in 2015 and 3.73% in 2017. Significant differences were recorded between the harvest years in terms of sucrose content ($p < 0.01$). The maximum sucrose value required under the Turkish Food Codex Notification on Honey [23] is 5%, and our findings were thus below this threshold. None of the honey samples in this study contained maltose, and this finding is thought to be an important criterion for pine honey. The sugar values in this research, except for maltose, are similar to those in previous studies conducted with different pine honeys [7, 30, 37].

Chemometric analysis

Principal component analysis (PCA) to determine the components that best explain the pine honey samples produced periodically in 47 different stations in three different regions, according to moisture, electrical conductivity, optical rotation angle, free acidity, color, proline, diastase, fructose/glucose ratio, and C4 sugar ratio measurements was conducted.

It was found to be important for the differentiation of pine honeys in terms of the parameters examined. The results
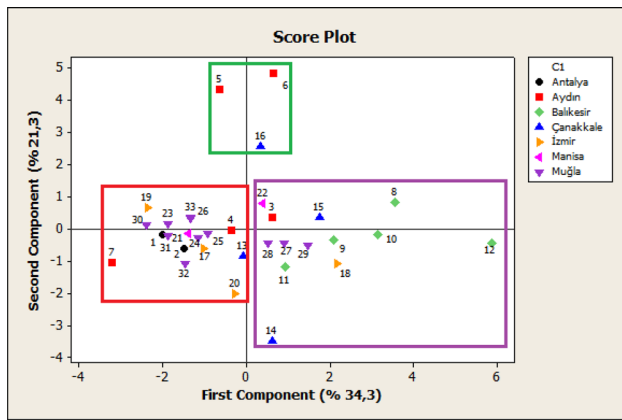
Fig. 2 Score plot graphic in terms of PC1 and PC2 in pine honey samples

showed that the first principal component (PC1) constituted 34.3% of the total variance and the second principal component (PC2) constituted 21.3%.

According to the parameters, it was observed that Turkish pine honey can be grouped on the first two principal components.

The score graph and loading plots are displayed in Figs. 2 and 3. It was determined that the pine honey samples were divided into three groups according to the production regions in terms of PC1 and PC2. While most of the pine honey produced in the Southwest Anatolian region clustered together, it was observed that it became different toward the north of the Aegean. It is observed that some regions of particularly Balıkesir and Çanakkale differ, and the sample of pine honey (sample 12) produced in Kepsut district of Balıkesir, which is adjacent to the Marmara region, differs from other stations. When Fig. 2 is examined, the two high-altitude stations of Aydın province (5 and 6) and the station (16) produced in the Çanakkale high-altitude region are clustered together. These data show that pine honey produced
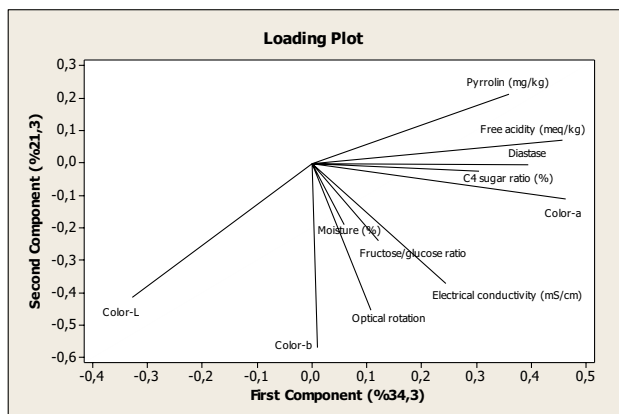
Fig. 3 Loading plot graphic in terms of PC1 and PC2 in pine honey samples
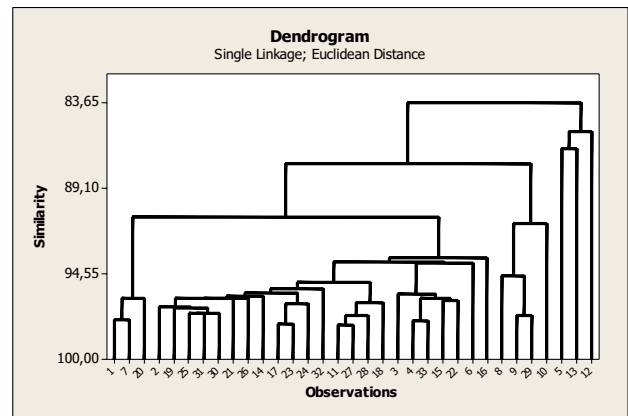

Fig. 4 Dendrogram results obtained by Euclidean distance and Ward Linkage method

in areas higher than 750 m altitude differs in terms of some parameters.

The dendrogram of the hierarchical cluster analysis (HCA) used to investigate the similarities of the pine honey samples produced at the stations with the highest pine honey production potential in seven different regions where pine honey is produced in Turkey is presented in Fig. 4. Physicochemical analysis results of all pine honey samples investigated were used in cluster analysis. In Fig. 4, the vertical axis shows the intercluster similarities and the horizontal axis shows the samples forming the clusters. When Fig. 4 is examined, it is seen that there are two main clusters. In the first main cluster, Aydın consists of samples 5, 12, and 13 (north Aegean and high-altitude station), and the second main cluster consists of other pine honey samples examined. In general, dendrogram analysis results were found to be compatible with PCA results.

Conclusions

In this study, performed under controlled conditions with no adulteration permitted, 373 samples of pine honeys from 47 locations were investigated. Our results were showed that all physicochemical parameters, except for C4% sugar, exhibited similar properties to those of pine honeys from other countries, as well as honeydew honey. The most important finding of this study is that C4% sugar is not an appropriate determining the quality of Turkish pine honey.

When the chemometric analysis results of the physicochemical analysis results of pine honey, which is the subject of this research and produced in a controlled manner from regions with high production potential in Turkey, are examined, it has been concluded that the production regions cause partial differentiation in the physicochemical properties of pine honey, and this may be caused by the color, optical rotation, electrical conductivity, and proline parameters.

Declarations

Conflict of interest There is no conflict of interest between the authors.

Ethical approval It is not a study that requires an ethics committee decision.

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