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Quality Profile of Several Monofloral Romanian Honeys

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Abstract: The objective of this research was to evaluate some quality-defining physicochemical parameters (moisture, specific gravity, pH, free acidity, ash, electrical conductivity, total phenols, and total flavonoids content, K, Ca, Mg, Na, and P) of seven Romanian monofloral honeys (linden, acacia, rapeseed, sunflower, mint, raspberry, and chestnut) collected in 2017. The investigated quality parameters are mainly within the recommended limits set by standards for honey. Sample analyses indicate the presence of antioxidants, such as TPC (17.9–73.2 mg GAE/100 g) and TFC (0.84–4.81 mg QE/100 g), and high amounts of K (101–1462 mg kg^{-1}), Ca (58.3–167.5 mg kg^{-1}), Mg (24.8–330.6 mg kg^{-1}), Na (94.5–233.3 mg kg^{-1}), and P (34.1–137.2 mg kg^{-1}). The Pearson's correlations between some parameters (such as color/TFC, color/Mg, color/P, EC/Ash, mm Pfund/TFC, TPC/TFC, K/Ash, P/Mg), together with PCA, HCA, and ANOVA statistics, highlight three main factors that explain the variability in the dataset and could be attributed to stability, mineral, and color/antioxidant contributions. FTIR spectra confirm the authenticity of all the monofloral honeys. The results and data processing confirm the influence of environmental elements (soil, water, air) on the honey composition and highlight the quality of honey, as a complete food and a therapeutic product.

Keywords: honey; phenols; flavonoids; minerals; FTIR; Pearson's correlation

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1. Introduction

Honey is considered one of the healthiest foods because any change of the environment quality produces a negative impact on the health of bees. Bees have important contributions to pollination, not only of wild plants that have nectar, but also of cultivated ones. Along with other pollinators, bees play an important role in maintaining biodiversity [1]. One of the measures to preserve biodiversity is to keep bees safe. Healthy bees have an impact over the quality of products of the hive. Honey is one of the main products of the hive. In Romania, the diversity of melliferous plants as well as those that are cultivated ensures a diversified production of polyfloral and especially monofloral honey [2]. Monofloral honey is a type of honey that comes mostly from the nectar of a single plant species, is more valuable, and has a higher price compared to the polyfloral honey [3]. It is known that the quality properties of honey differ from one year to another, and responsible for this, first, are the climatic variations of the last years and other factors such as the pastoral regions, environmental quality (soil, water, air), and mainly the botanical origin [4–7]. The nutritional and therapeutic quality of honey has been known for a long time, and therefore its use in different fields (nutrition, medicine, cosmetics, etc.) has been continuously increasing.

Honey contains many substances (mainly sugars, water, organic acids, proteins, vitamins, phenolic compounds, minerals, pigments, etc.) that, in perfect harmony, give it its known properties [4,8–10]. Qualitatively, honey must comply with a series of legislative requirements, not more than 20% moisture content, not less than 60 g/100 g sugars' content, free acidity not more than 50 milliequivalents acid per 1000 g, etc. [11]. The moisture level

is important, because with a higher content of moisture, honey can ferment and other parameters such as viscosity, taste, and odor change and contribute to the depreciation of honey [10]. All the studied European honeys were acidic, and a low pH inhibits the growth of microorganisms. Free acidity is considered a freshness indicator and the flavors of honey are due to the presence of organic acids [2,12–16]. Honeydew and blossom honey differ through composition. The value of electrical conductivity established by legislation [11] delimits the botanical origin of blossom honey from honeydew, and it could be considered a criterion for determining a possible honey adulteration. This parameter is correlated with ash, salts, concentrations of mineral elements, etc. [8,10,12,17,18]. Other constituents of honey that have a special role are polyphenols and flavonoids. The quantity and the type of these antioxidant compounds in honey come mainly from flowers, which give this beehive product its antioxidant properties, and are responsible for its therapeutic qualities [19,20]. Each mineral and trace element in honey varies depending on the geographical as well as the floral origin. Minerals in honey come from the environment, mainly from the soil, from which plants absorb them. Plants absorb both essential minerals for human health (K, Ca, Mg, Na, P, Cu, Mn, Fe) and toxic ones (Pb, Cd, Hg). The traceability of these mineral elements from soil to honey can negatively influence the quality of the hive products. The presence and quantity of minerals in honey could reflect the quality and degree of pollution, which is why honey has also been considered as a possible indicator of the environment quality [9]. Many researchers have shown some correlations between compounds of honey that could help to identify some common characteristics of different types of honey from different geographical areas [21–28].

The analyses carried out on honey involve the structural destruction of the sample, the consumption of chemical reagents, and consequently, take a long time to obtain viable results. In recent years, Fourier transform infrared (FTIR) spectroscopy has been used in honey research. This is a nondestructive analytical method used to scan, to identify, or to highlight certain substances or chemical groups present in the composition of the tested samples and the chemical properties of the samples. FTIR spectroscopy yields valuable information on the quality of honey and is important to verify the authenticity of the honey and its possible adulteration [29–31]. The technique was applied on different types of honey samples to determine the botanical or geographical origin, and to identify some chemical compounds that define the specific quality of honey, such as carbohydrates (fructose, glucose, sucrose) [32–35].

The aim of this study was to evaluate the quality of some monofloral honey types produced in Romania by using classical physicochemical methods and to show the similarities or molecular differences with a nondestructive method—FTIR spectroscopy.

2. Materials and Methods

2.1. Honey Samples

The honey samples, from *Apis mellifera* species, collected in 2017, come from two companies that collect, process, and then sell the honey (producers 1 and 2) and from four private beekeepers (producers 3, 4, 5, and 6) (Table 1). The honey samples originate from mainly the eastern part of Romania, as well as the northwestern and southeastern parts (Figure 1).

The botanical origin of raw honey samples was established by a company that purchases the honey from the beekeepers, by using melissopalynological methods. Three jars of 750 g each were collected from every type of honey. All samples were kept in the dark at laboratory temperature (22 ± 2 °C). The crystallized samples were liquefied at 40 °C in a water bath (Memmert GMBH—Schwabach, Germany), homogenized, and filtered through gauze.

Table 1. Sample description.

Sample	Producer	Location	Geographical Location	Sample	Producer	Location	Geographical Location
L1	1	Barnova Iasi	47°05′32″ N 27°38′14″ E	L3	3	Popesti Iasi	47°08′42″ N 27°15′33″ E
A1	1	Lunca Prutului Iasi	47°27′0″ N 27°33′0″ E	A3	3	Baltati Iasi	47°13′42″ N 27°06′32″ E
RP1	1	Barnova Iasi	47°05′32″ N 27°38′14″ E	RP3	3	Baltati Iasi	47°13′42″ N 27°06′32″ E
SF1	1	Barnova Iasi	47°05′32″ N 27°38′14″ E	SF3	3	Popesti Iasi	47°08′42″ N 27°15′33″ E
M1	1	Danube Delta Tulcea	45°20′ N 29°30′ E	L4	4	Aroneanu Iasi	47°12′50″ N 27°36′00″ E
RB1	1	Bistrita mountains	47°12′, 25°67′ E	A4	4	Aroneanu Iasi	47°12′50″ N 27°36′00″ E
C1	1	Satu Mare county	47°47′24″ N 22°53′24″ E	L5	5	Tomesti Iasi	47°07′07″ N 27°42′48″ E
L2	2	Raducaneni Iasi	46°57′33″ N 27°58′41″ E	A5	5	Tomesti Iasi	47°07′07″ N 27°42′48″ E
RP2	2	Raducaneni Iasi	46°57′33″ N 27°58′41″ E	L6	6	Roscani Iasi	47°26′18.12″ N 27°25′42.78″ E
M2	2	Danube Delta Tulcea	45°20′ N 29°30′ E	A6	6	Roscani Iasi	47°26′18.12″ N 27°25′42.78″ E
RB2	2	Dobrovat Iasi	46°57′55″ N 27°43′18″ E				

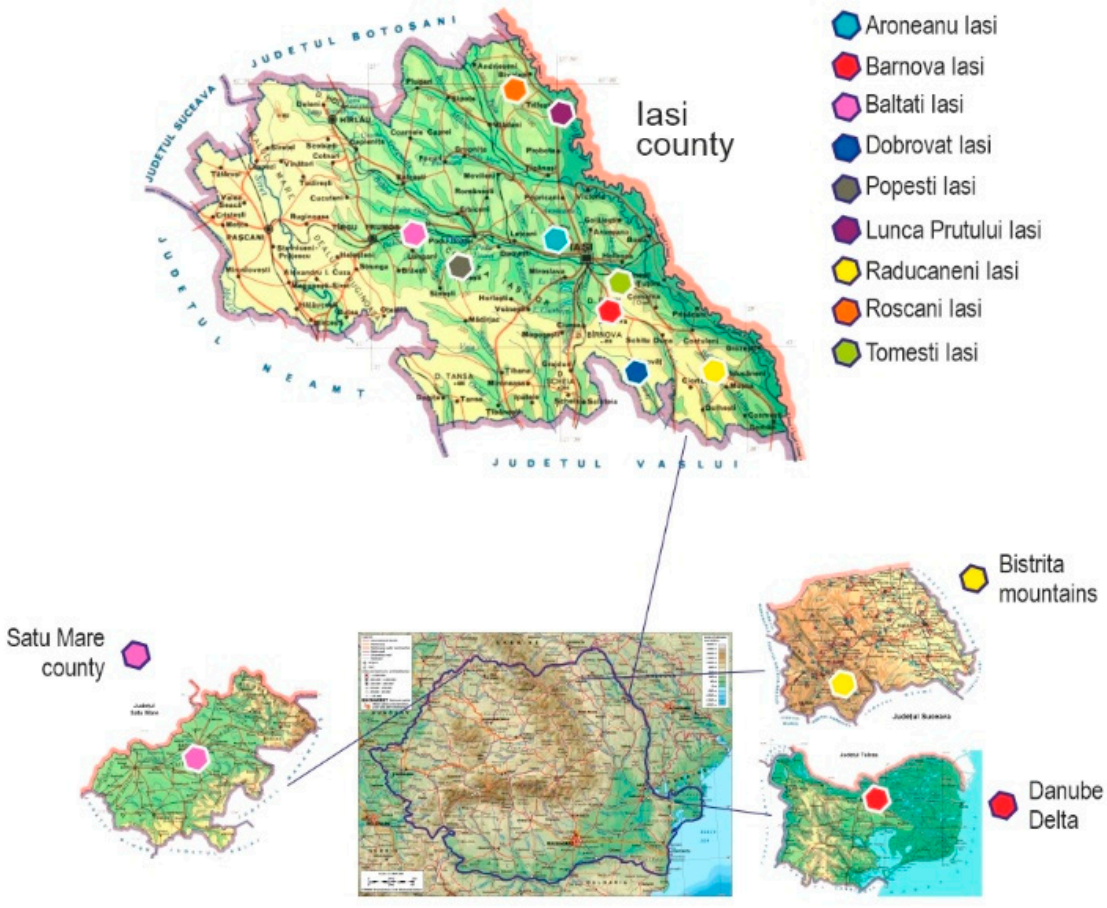


Figure 1. Map showing the locations where the honey samples were produced.

2.2. Pfund Value and Color

The Pfund value and the color of honey samples were determined using the method described by Ratiu et al. [17,26,36]. A 50% (*w/v*) honey aqueous solution was centrifuged at 3200 rpm (UNIVERSAL 320 HETTICH centrifuge, Hettich GMBH—Tuttlingen, Germany) and the absorbance was measured at 635 nm using a Shimadzu UV-1700 Pharma Spec spectrophotometer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). The absorbance units were converted to mm Pfund with the relation:

$$\text{Pfund (mm)} = -38.7 + 371.39 \times \text{Abs} \quad (1)$$

where Pfund represents the honey color value in the Pfund scale (mm), and Abs is the absorbance at 635 nm.

2.3. Refractive Index, Moisture, Solid Substances, and Specific Gravity

Moisture content (M), expressed as a percentage, was taken from the table of correspondence between the water content and the refractive index at 20 °C [37] after reading the refractive index on an ABBÉ Kruss AR 2008 refractometer (Kruss Scientific GMBH, Hamburg, Germany) and applying the appropriate temperature correction. Solid substances' content (SS), expressed as a percentage, was calculated as the difference between 100 and the moisture content. Specific gravity was determined by the gravimetric method, using a pycnometer device. The results were expressed in g/cm^3 [38,39].

2.4. pH and Free Acidity

The pH values were measured in a honey solution (10 g of honey in 75 mL of distilled water) using the MULTI 3320 multiparameter (WTW GMBH, Weilheim, Germany). Free acidity was determined by titration with 0.1 N NaOH (Chemical Company, Iasi, Romania) of a honey solution (10 g of honey in 75 mL of distilled water) using phenolphthalein (Chimreactiv, Bucuresti, Romania) as a color indicator. The free acidity was expressed in g/cm^3 [10,37,39].

2.5. Ash and Electrical Conductivity

Ten grams of each honey sample were weighed in porcelain crucibles and were calcinated in a furnace (Nabertherm B180, Nabertherm GMBH, Lilienthal, Germany), and the ash content was expressed in g/100 g. The 20% solution (the mass of honey was calculated as dry matter) with ultrapure water (Barnstead EASY PURE II, Thermo Fisher Scientific Co. Ltd.; Marietta, OH, USA) was measured with the MULTI 3320 multiparameter (WTW GMBH, Weilheim, Germany). The electrical conductivity was expressed in $mS\ cm^{-1}$ [37,39].

2.6. Total Phenols Content and Total Flavonoids Content

The extraction of the total phenols and total flavonoids was carried out with an alcoholic solution (1:1 equal parts of methanol (Merck KGaA, Darmstadt, Germany) and acidified water (deionized water at pH = 2 with HCl (Merck KGaA, Darmstadt, Germany)). The 10% honey solution obtained by mixing the corresponding amount of honey with the prepared alcoholic solution was homogenized and filtered through filter paper. An aliquot of filtered honey solution was mixed with 0.2 mL of Folin–Ciocalteu's phenol reagent (Merck KGaA, Darmstadt, Germany) for 5 min and 75 g/L of Na_2CO_3 (Merck KGaA, Darmstadt, Germany) was added to a total volume of 10 mL. The solution was spectrophotometrically analyzed at 742 nm, after incubating in the dark at room temperature for 30 min (Shimadzu UV-1700 Pharma Spec, Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). The calibration curve was linear ($y = 0.0993x + 0.0741$; $R^2 = 0.9991$) in the concentration range of 2–12 mg L^{-1} gallic acid (Merck KGaA, Darmstadt, Germany). The total phenols content was expressed as mg of gallic acid equivalents (GAE)/100 g [10,40].

Total flavonoids were determined in the same alcoholic solution of honey prepared for the determination of total polyphenols. Equal volumes of 2% $AlCl_3$ (Merck KGaA, Darmstadt, Germany) and honey solution were mixed, and after 10 min, the absorbance was measured at 430 nm. A standard solution of quercetin (Sigma-Aldrich, Saint Louis, MO, USA) was prepared and used to obtain the calibration curve (concentration range 0.5–5 mg L^{-1}; y = 0.01330x + 0.0111; R^2 = 0.9998). The total flavonoids content was expressed as mg of quercetin equivalents (QE)/100 g [26,40].

2.7. Mineral Elements (K, Ca, Mg, Na, and P)

To determine the mineral elements' content, the ash resulting from the calcination of the samples was moistened with ultrapure water, evaporated in a sand bath, and after calcination for 6 h, treatment with 6 M HCl, and heating to evaporate the acid, the residue was dissolved in 0.1 M nitric acid. The extracts were then filtered, and ultrapure water was added to a total volume of 25 mL. The presence of possible contaminants during the digestion process was controlled using blanks. Phosphorus was spectrophotometrically determined with molybdovanadate reagent at 430 nm (Shimadzu UV-1700 Pharma Spec, Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) [41]. The calibration curve was linear in the concentration range of 5–50 mg L^{-1} (y = 0.0209x + 0.0150; R^2 = 1.000). Ca and Mg were determined by flame atomic absorption spectrometry (Ca: y = 0.0264x + 0.0140, R^2 = 0.9995, 1–10 mg L^{-1}; Mg: y = 0.3997x + 0.0253, R^2 = 0.9991, 0.1–0.5 mg L^{-1}), and Na and K were determined by flame atomic emission spectrometry (Na: y = 0.0970x + 0.0017, R^2 = 0.9966, 1–10 mg L^{-1}; K: y = 0.1010x + 0.0128, R^2 = 0.9988, 1–10 mg L^{-1}) (Analytik Jena novAA 350, Analytik Jena GmbH, Jena, Germany).

2.8. FTIR Analysis

Infrared spectra were obtained by using a Jasco FT/IR-660 Plus Fourier Transform Infrared Spectrometer (Tokyo, Japan). The honey samples were liquefied at 40 °C and homogenized. A small quantity of the honey samples was incorporated into a KBr (Sigma-Aldrich, Darmstadt, Germany) pellet. Spectral measurements were recorded in the wavenumber over a domain from 4000 to 400 cm^{-1} with 32 scans, with a resolution of 4 cm^{-1} [30].

From the FTIR analysis, the raw data of 21 honey samples were saved in a file with ".jws" and ".txt" extensions. In each obtained spectrum, the transmittance vs. wavenumber was plotted. With the OriginPro 2022 software, the spectra of the honey samples were obtained and graphically displayed.

2.9. Statistical Analyses

All analyses were performed in triplicate. Statistical analyses were performed using the software package STATISTICA 12.0 (StatSoft Inc, Tusla, OK, USA). Correlations between the investigated parameters were tested using Pearson's correlation coefficient. Principal component analysis and hierarchical cluster analysis were used to obtain an overview of physicochemical parameters' contributions.

3. Results

3.1. Physicochemical Analyses

Figure 2 shows the color differences of the 21 honey samples and their classifications according to the Pfund scale.

In Table 2, the values of mm Pfund, refractive index, moisture, solid substances' content, and specific gravity are presented.

The results for pH, free acidity, ash, electrical conductivity, total phenols content, and total flavonoids content are presented in Table 3.

Table 4 lists the concentrations of mineral elements in the analyzed honeys.

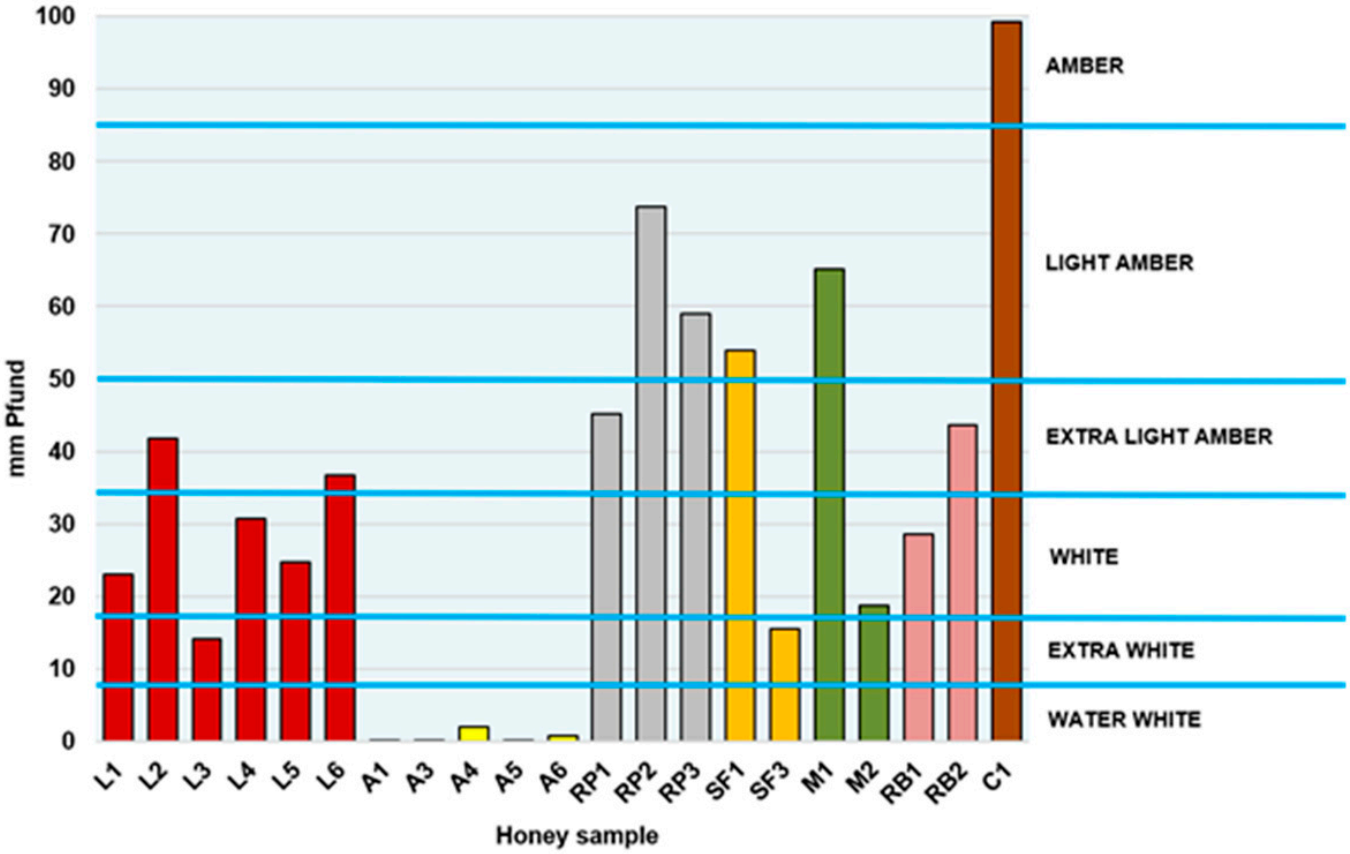


Figure 2. The color of the honey samples according to the Pfund scale.

Table 2. Parameter values (mm Pfund, refractive index, moisture, solid substances' content, and specific gravity) of seven types of honey.

Parameter	Descriptive Statistics	Type							ANOVA
		Linden 6 Samples	Acacia 5 Samples	Rapeseed 3 Samples	Sunflower 2 Samples	Mint 2 Samples	Raspberry 2 Samples	Chestnut 1 Sample	
mm Pfund	Min–Max Mean ± SD CV	23.1–41.8 28.6 ± 9.94 34.81	0.1–2.0 0.6 ± 0.81 132	45.2–73.7 59.3 ± 14.30 24.12	15.5–53.9 34.7 ± 27.14 78.27	18.8–65.2 42.0 ± 32.83 78.17	28.5–43.7 36.1 ± 10.75 29.77	98.3–100.2 99.2 ± 0.59 0.59	***
RI	Min–Max Mean ± SD CV	1.486–1.494 1.489 ± 0.00 0.20	1.488–1.493 1.491 ± 0.00 0.15	1.486–1.492 1.488 ± 0.00 0.24	1.488–1.493 1.490 ± 0.00 0.21	1.490–1.491 1.490 ± 0.00 0.05	1.490–1.492 1.491 ± 0.00 0.09	1.497–1.498 1.497 ± 0.00 0.01	ns
M %	Min–Max Mean ± SD CV	17.0–20.4 18.9 ± 1.18 6.24	17.5–19.5 18.3 ± 0.92 5.04	17.8–20.4 19.5 ± 1.44 7.36	17.6–19.3 18.4 ± 1.24 6.75	18.3–18.7 18.5 ± 0.29 1.55	17.8–18.7 18.3 ± 0.64 3.49	15.9–16.0 15.9 ± 0.03 0.21	ns
SS %	Min–Max Mean ± SD CV	79.6–83.0 81.1 ± 1.18 1.46	80.5–82.5 81.7 ± 0.92 1.13	79.6–82.2 80.5 ± 1.44 1.78	80.7–82.4 81.6 ± 1.24 1.53	81.3–81.7 81.5 ± 0.29 0.35	81.3–82.2 81.8 ± 0.64 0.78	84.0–84.1 84.1 ± 0.03 0.04	ns
SG g/cm^3	Min–Max Mean ± SD CV	1.415–1.437 1.420 ± 0.01 0.55	1.421–1.434 1.428 ± 0.01 0.44	1.415–1.432 1.421 ± 0.01 0.68	1.422–1.434 1.428 ± 0.01 0.59	1.426–1.429 1.28 ± 0.00 0.14	1.426–1.432 1.429 ± 0.00 0.30	1.444–1.445 1.445 ± 0.00 0.02	ns

RI—refractive index, M—moisture, SS—solid substances, SG—specific gravity, SD—standard deviation, CV—coefficient of variation. Significant difference at: $p < 0.001$ (***), ns—not significant.

Table 3. Parameter values (pH, free acidity, ash, electrical conductivity, total phenols content, total flavonoids content) of seven types of honey.

Parameter	Descriptive Statistics	Type							ANOVA
		Linden 6 Samples	Acacia 5 Samples	Rapeseed 3 Samples	Sunflower 2 Samples	Mint 2 Samples	Raspberry 2 Samples	Chestnut 1 Sample	
pH	Min–Max Mean ± SD CV	3.81–5.08 4.59 ± 0.44 9.50	3.94–4.64 4.17 ± 0.27 6.49	3.95–4.17 4.06 ± 0.11 2.65	3.91–4.91 4.41 ± 0.71 16.09	3.99–4.77 4.38 ± 0.55 12.54	4.27–4.30 4.29 ± 0.02 0.50	4.63–4.67 4.65 ± 0.01 0.31	ns
FA meq kg^{-1}	Min–Max Mean ± SD CV	23.4–38.6 29.51 ± 5.20 17.64	12.8–25.4 19.8 ± 5.08 25.60	24.3–46.6 34.7 ± 11.21 32.30	35.6–50.1 42.9 ± 10.25 23.92	38.2–45.7 42.0 ± 5.33 12.71	25.8–42.0 33.9 ± 11.46 33.79	49.6–49.9 49.8 ± 0.11 0.23	**
Ash %	Min–Max Mean ± SD CV	0.199–0.471 0.33 ± 0.11 34.75	0.044–0.190 0.098 ± 0.06 64.12	0.061–0.114 0.088 ± 0.03 30.56	0.184–0.501 0.343 ± 0.22 65.45	0.199–0.213 0.206 ± 0.01 4.81	0.174–0.176 0.175 ± 0.00 0.81	0.459–0.502 0.483 ± 0.02 3.30	**
EC mS cm^{-1}	Min–Max Mean ± SD CV	0.358–0.692 0.594 ± 0.17 27.82	0.140–0.320 0.200 ± 0.08 40.36	0.209–0.252 0.2350.02± 9.73	0.469–0.699 0.584 ± 0.16 27.85	0.503–0.542 0.523 ± 0.03 5.28	0.294–0.378 0.336 ± 0.06 17.68	0.920–0.935 0.927 ± 0.01 0.57	***
TPC mg GAE/100g	Min–Max Mean ± SD CV	22.0–27.5 25.1 ± 2.42 9.63	13.7–23.1 17.9 ± 3.79 21.17	22.4–24.1 23.3 ± 0.87 3.72	21.10–23.90 22.50 ± 1.98 8.80	50.3–58.7 54.5 ± 5.93 10.89	28.2–33.5 30.9 ± 3.75 12.15	72.5–74.1 73.2 ± 0.51 0.70	***
TFC mg QE/100g	Min–Max Mean ± SD CV	1.48–2.56 1.81 ± 0.43 24.0	0.46–1.29 0.87 ± 0.32 36.18	2.29–2.87 2.43 ± 0.33 13.35	1.21–2.56 1.89 ± 0.95 50.64	2.12–3.62 2.87 ± 1.06 36.96	2.38–2.87 2.63 ± 0.35 13.20	4.21–5.27 4.81 ± 0.31 6.41	***

FA—free acidity, EC—electrical conductivity, TPC—total phenols content, TFC—total flavonoids content, SD—standard deviation, CV—coefficient of variation. Significant difference at: $p < 0.01$ (**), $p < 0.001$ (***), ns—not significant.

Table 4. Concentrations of mineral elements (K, Ca, Mg, Na, P) in seven types of honey.

Parameter	Descriptive Statistics	Type							ANOVA
		Linden 6 Samples	Acacia 5 Samples	Rapeseed 3 Samples	Sunflower 2 Samples	Mint 2 Samples	Raspberry 2 Samples	Chestnut 1 Sample	
K mg kg^{-1}	Min–Max Mean ± SD CV	404–1507 984 ± 476 48.45	75–213 131 ± 53.96 41.15	46–196 101 ± 82.76 82.08	455–1150 803 ± 491.44 61.24	415–697 556 ± 199.15 35.81	228–327 278 ± 70.00 25.23	1455–1467 1462 ± 1.71 0.12	**
Ca mg kg^{-1}	Min–Max Mean ± SD CV	31.7–429.8 167.5 ± 143 85.51	17.7–139.7 62.2 ± 53.41 85.82	24.3–84.3 58.3 ± 30.79 52.81	131.0–132.0 131.5 ± 0.71 0.54	122.6–171.9 147.3 ± 34.86 23.67	15.7–128.8 72.3 ± 79.97 110.69	155.7–173.1 164.0 ± 5.50 3.35	ns
Mg mg kg^{-1}	Min–Max Mean ± SD CV	39.4–96.9 59.4 ± 21.12 35.54	10.8–64.6 24.8 ± 22.52 90.80	33.3–58.6 46.2 ± 12.66 27.38	50.8–74.3 62.6 ± 16.62 26.57	29.5–78.2 53.9 ± 34.44 63.95	70.9–72.8 71.9 ± 1.34 1.87	320.2–339.0 330.6 ± 1.23 0.37	***
Na mg kg^{-1}	Min–Max Mean ± SD CV	97.9–223.1 169.4 ± 49.41 29.18	28.4–292.6 94.5 ± 113.23 119.85	45.0–128.3 98.0 ± 46.03 46.99	129.7–247.0 188.4 ± 82.94 44.04	172.5–181.2 176.9 ± 6.15 3.48	110.1–156.1 133.1 ± 32.53 24.44	216.0–245.2 233.3 ± 5.79 2.48	ns
P mg kg^{-1}	Min–Max Mean ± SD CV	29.8–52.9 42.1 ± 9.68 22.98	21.8–47.3 34.1 ± 9.69 28.41	39.5–50.5 45.4 ± 5.54 12.21	40.1–52.5 46.3 ± 8.77 18.94	43.5–74.5 59.0 ± 21.92 37.15	44.2–79.4 61.8 ± 24.89 40.28	125.6–145.1 137.2 ± 1.05 0.76	***

SD—standard deviation, CV—coefficient of variation. Significant difference at: $p < 0.01$ (**), $p < 0.001$ (***), ns—not significant.

Statistical differences based on the mean values of the determined parameters between all types of analyzed samples (one-way ANOVA) are listed in Tables 2–4. In Table 5, the statistical differences for the investigated quality parameters for pairs of different honey types are summarized.

3.2. FTIR Spectra

In Table 6, the band wavelengths of honey samples' spectra are presented. Figures 3 and 4 show the FTIR spectra of the analyzed honey samples in the 4000–400 cm^{-1} and 1700–400 cm^{-1} spectral regions.

3.3. Correlation and Multivariate Statistical Analysis

The correlations between all studied parameters are summarized in Table 7.

Table 5. Analysis of variance (ANOVA) for the determined quality parameters (pairs of honey types).

	mm Pfund	RI	M	SS	SG	pH	FA	Ash	EC	TPC	TFC	K	Ca	Mg	Na	P
L-A	***	ns	ns	ns	ns	ns	*	**	***	**	**	**	ns	*	ns	ns
L-RP	**	ns	ns	ns	ns	ns	ns	**	**	ns	ns	*	ns	ns	ns	ns
L-SF	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
L-M	ns	ns	ns	ns	ns	ns	*	ns	ns	***	ns	ns	ns	ns	ns	ns
L-RB	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
L-C	**	ns	ns	ns	ns	ns	*	ns	ns	***	**	ns	ns	***	ns	***
A-RP	***	ns	ns	ns	ns	ns	*	ns	ns	ns	***	ns	ns	ns	ns	ns
A-SF	*	ns	ns	ns	ns	ns	**	ns	**	ns	ns	*	ns	ns	ns	ns
A-M	*	ns	ns	ns	ns	ns	**	ns	**	***	**	**	ns	ns	ns	ns
A-RB	***	ns	ns	ns	ns	ns	ns	ns	ns	**	**	*	ns	*	ns	ns
A-C	***	ns	ns	ns	ns	ns	**	**	**	***	***	***	ns	***	ns	***
RP-SF	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns
RP-M	ns	ns	ns	ns	ns	ns	ns	**	***	**	ns	*	ns	ns	ns	ns
RP-RB	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns
RP-C	ns	ns	ns	ns	ns	*	ns	**	**	***	*	**	ns	**	ns	**
SF-M	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
SF-RB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SF-C	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	*	ns	ns
M-RB	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns
M-C	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
RB-C	ns	ns	ns	ns	ns	*	ns	**	ns	ns	ns	*	ns	**	ns	ns

Significant difference at: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), ns—not significant.

Table 6. Positions of absorption bands for the monofloral honey samples analyzed by FTIR.

Spectral Range	FT-IR Wavenumber (cm^{-1})						
	Linden	Acacia	Rapeseed	Sunflower	Mint	Raspberry	Chestnut
D1	3377–3416	3393–3415	3383–3398	3396–3408	3376–3388	3370–3416	3369
D2	2933–2934	2934–2935	2932–2933	2922–2933	2933	2929–2933	2933
D3	2117–2120	2118–2119	2115–2118	2120–2121	2116–2118	2116–2117	2119
D4	1641–1647	1636–1647	1638–1646	1637–1640	1637–1639	1640–1647	1638
D5	1452–1453	1452–1454	1453–1454	1450–1454	1452–1453	1453	1454
D6	1415–1416	1415–1418	1415–1417	1415–1418	1414–1416	1415–1416	1416
D7	1342–1350	1348–1350	1340–1342	1348	1342–1344	1339–1345	1343
D8	1256–1258	1256–1257	1256	1257	1255–1256	1255–1259	1256
D9	1144–1146	1144–1146	1144	1144–1145	1144	1144–1145	1145
D10	1055–1057	1054–1056	1056	1056	1056	1054–1057	1056
D11	919–920	919–920	918–919	919	919	919	919
D12	866–867	867	866–867	867	867–868	867	867
D13	819	819–819	818	818	818	818–819	819
D14	778–779	778–779	778	778	778	778–779	778

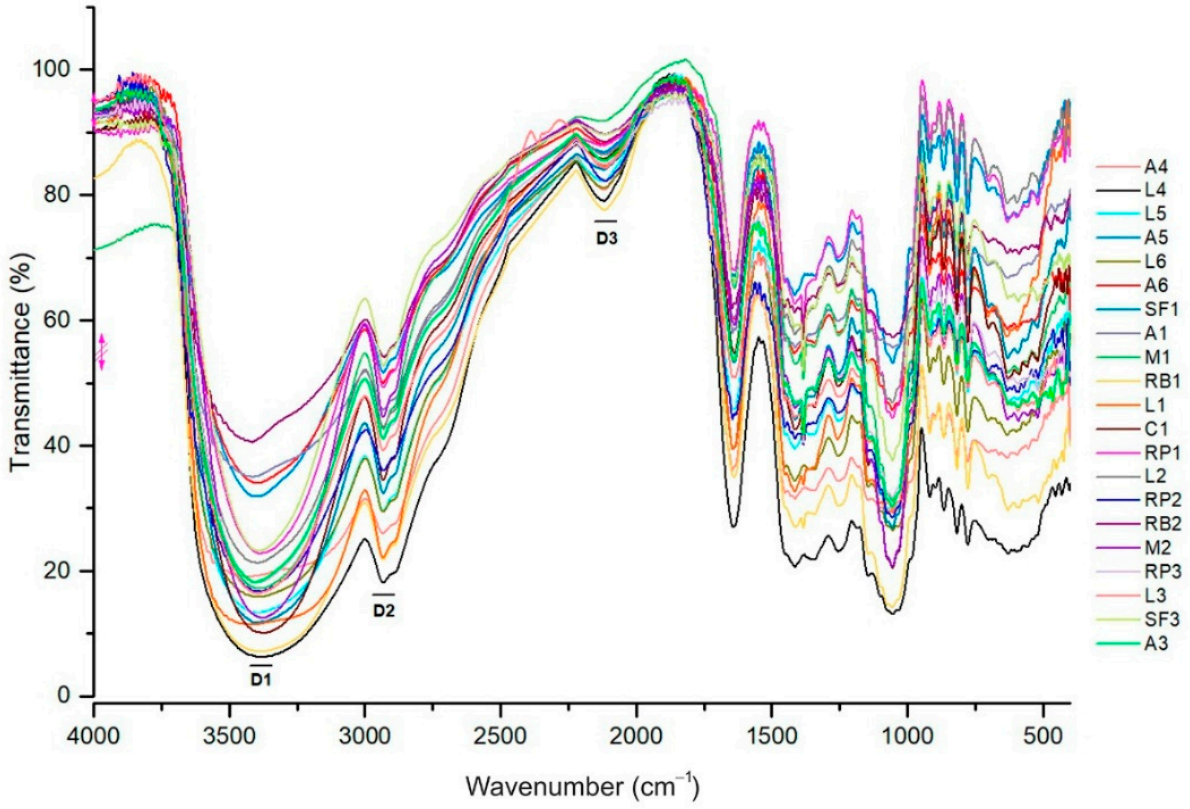


Figure 3. FTIR spectra of all studied honey samples in the spectral region from 4000 to 400 cm^{-1}.

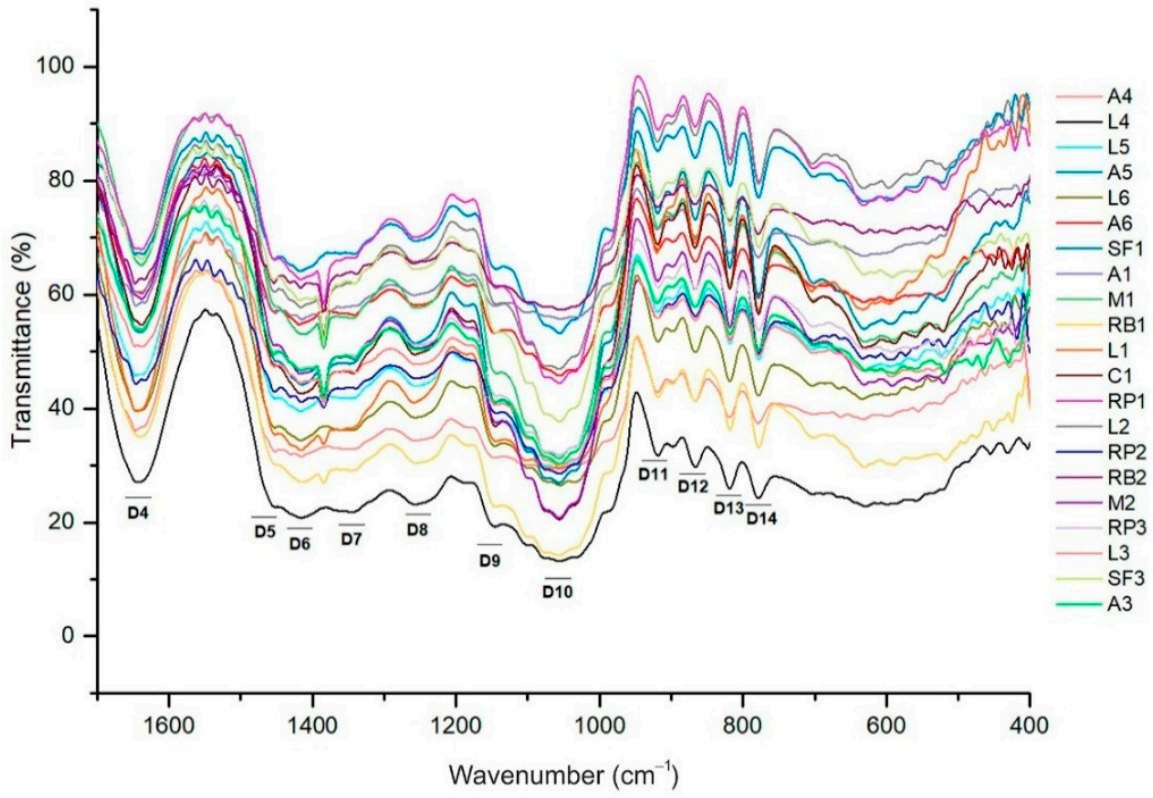


Figure 4. FTIR spectra of all studied honey samples in the spectral region from 1700 to 400 cm^{-1}.

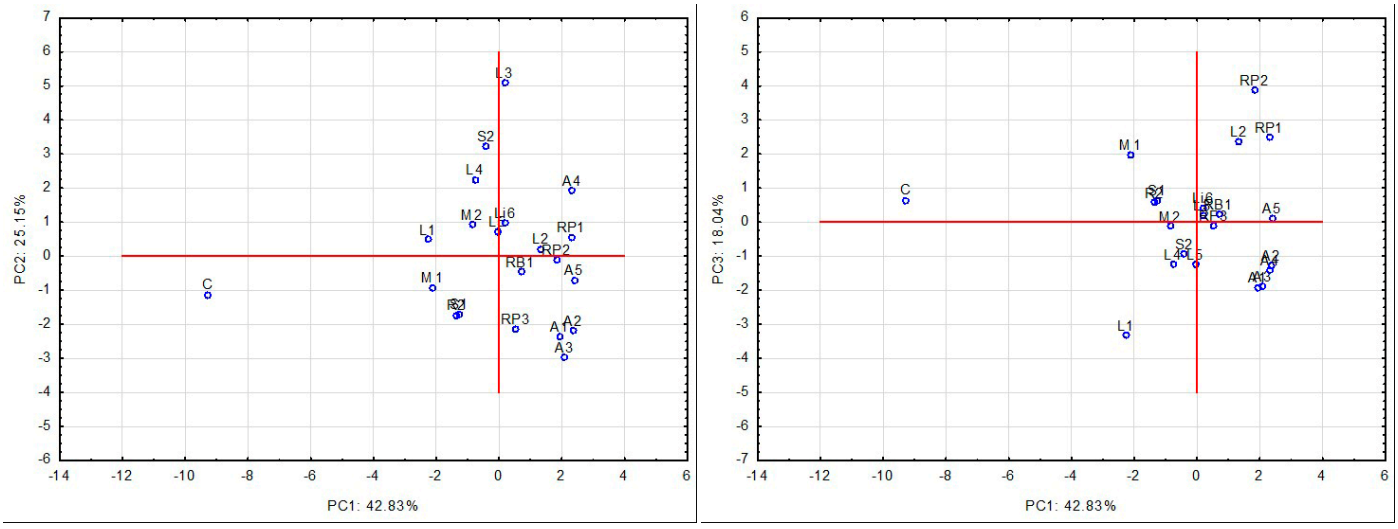
Table 7. Pearson's correlation coefficients between the investigated honey parameters (significant at: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)). The values of significant correlation coefficients are marked in bold.

	RI	M	SS	Color	SG	pH	FA	Ash	EC	TPC	TFC	K	Ca	Mg	Na	P
RI	1.00															
M	**−0.99** ***	1.00														
SS	**0.99** ***	**−1.00** ***	1.00													
Color	0.61	−0.61	0.61	1.00												
SG	**0.99** ***	**−0.99** ***	**0.99** ***	0.61	1.00											
pH	0.62	−0.63	0.63	0.41	0.62	1.00										
FA	0.55	−0.55	0.55	**0.82** *	0.55	0.52	1.00									
Ash	0.71	−0.72	0.72	0.58	0.71	**0.93** **	0.68	1.00								
EC	0.74	−0.74	0.74	0.68	0.74	**0.92** **	**0.76** *	**0.97** ***	1.00							
TPC	**0.79** *	**−0.79** *	**0.79** *	**0.78** *	**0.80** *	0.59	**0.75** *	0.61	**0.75** *	1.00						
TFC	0.73	−0.73	0.73	**0.95** ***	0.73	0.50	**0.83** *	0.61	0.72	**0.90** **	1.00					
K	0.70	−0.71	0.71	0.60	0.70	**0.95** ***	0.65	**0.98** ***	**0.98** ***	0.66	0.63	1.00				
Ca	0.45	−0.46	0.46	0.40	0.45	**0.93** **	0.58	**0.85** *	**0.89** **	0.59	0.46	**0.90** **	1.00			
Mg	**0.91** **	**−0.91** **	**0.91** **	**0.84** *	**0.91** **	0.64	0.67	**0.77** *	**0.81** *	**0.82** *	**0.88** **	**0.78** *	0.51	1.00		
Na	0.71	−0.72	0.72	0.63	0.72	**0.90** **	**0.81** *	**0.95** ***	**0.98** ***	**0.75** *	0.70	**0.94** **	**0.89** **	0.75	1.00	
P	**0.91** **	**−0.91** **	**0.91** **	**0.86** *	**0.91** **	0.59	0.72	0.71	**0.78** *	**0.89** **	**0.93** **	0.72	0.47	**0.98** ***	0.73	1.00

The results of the principal component analysis are shown in Table 8 and Figure 5. In Figure 6, the hierarchical dendrogram of cluster analysis is presented.

Table 8. Loadings and explained variance (%) for the extracted principal components for the analyzed honey samples. The values of significant correlation coefficients are marked in bold.

Variable	PC 1	PC 2	PC 3
RI	**0.98**	0.03	0.15
M	**−0.99**	−0.03	−0.15
SS	**0.99**	0.03	0.15
Color	0.05	0.00	**0.93**
SG	**0.98**	0.02	0.15
pH	0.12	**0.91**	−0.23
FA	−0.06	0.03	**0.88**
Ash	0.00	**0.94**	0.19
EC	0.12	**0.90**	0.36
TPC	0.31	0.31	**0.73**
TFC	0.12	0.02	**0.98**
K	0.15	**0.88**	0.18
Ca	−0.31	0.66	0.04
Mg	0.31	0.47	0.70
Na	0.00	**0.81**	0.10
P	0.33	0.16	**0.86**
Eigenvalue	6.85	4.02	2.89
% Total variance	42.83	25.15	18.04
Cumulative %	42.83	67.97	86.01



(**a**) (**b**)

Figure 5. Graphical representation of PC for the analyzed honey samples: (**a**) scores for PC2 vs. PC1 and (**b**) scores for PC3 vs. PC1.

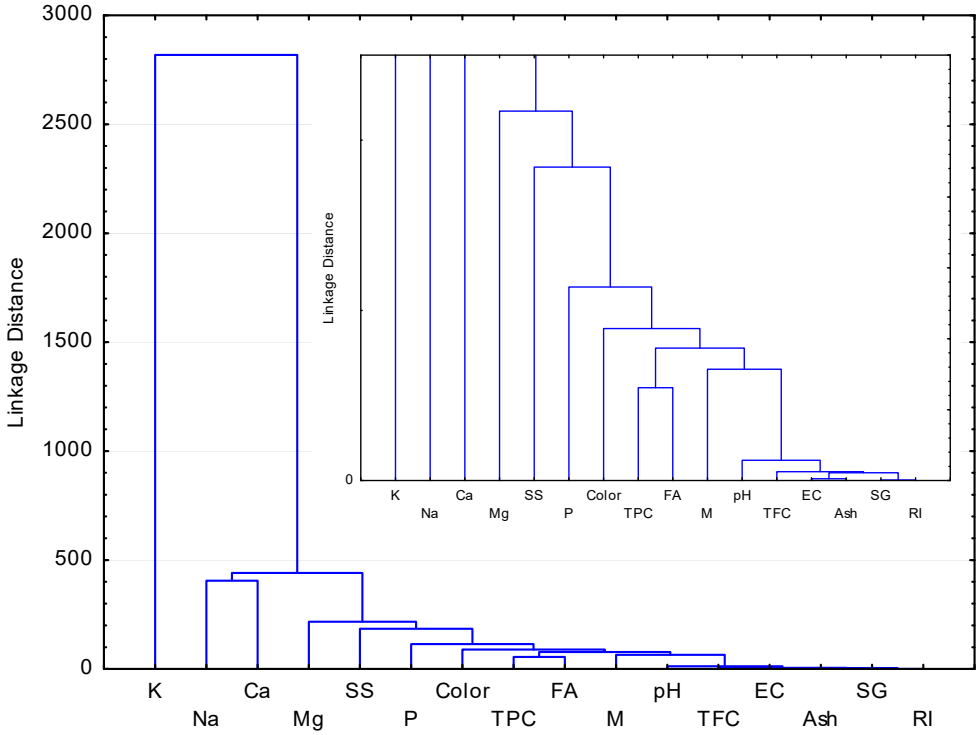


Figure 6. The hierarchical dendrogram of cluster analysis for the analyzed honey samples based on the determined quality parameters.

4. Discussion

4.1. Color

The first opinion on the quality of a food product for a consumer is the color, and subsequently the flavor and taste. Consumer preferences on honey vary, some prefer light-colored honey and others dark-colored, and these preferences can affect the price [4]. The colors of honey are grouped into seven categories: water white, extra white, white, extra light amber, light amber, amber, and dark amber, without considering the variations of shades that they can have. There are several methods to determine the color of honey, such as Pfund, Lovibond, and Jack's scale color grader [42]. The color of honey is directly influenced by various factors, such as water content and some chemical compounds (phenolic, carotenoids, minerals), pollen floral types, and geographical origin, and indirectly by technological conditions (temperature, processing/handling/storage), time, etc. [8,42–44]. In this study, the average honey color values ranged from 0.1 mm Pfund for acacia honey to 100.2 mm Pfund for chestnut honey (Table 1). The color of linden honey varied between 23.1 and 41.80 mm Pfund (extra white–extra light amber). High differences were found for mint honey of 46.4 mm Pfund and of 38.4 mm Pfund for sunflower honey. Similar high differences were found by Ratiu et al. for rapeseed and sunflower honey from Poland (Table 9) [17]. All acacia samples have low values of mm Pfund, such as those observed by Flanjak et al. for acacia honey from Croatia [45]. It is known that chestnut honey is dark-colored, as also shown in other studies when higher values were reported than those found in this study [5,42] (Table 9). The results in the present study showed the highest concentration of antioxidant compounds in the dark-colored honey. Considering the variability in the global dataset, the ANOVA indicated significant differences at the $p < 0.001$ level (Table 2), mainly based on the statistically significant differences between acacia honey and the other monofloral honey types (Table 5) and also between chestnut and linden honeys.

Table 9. Comparative results on color intensity (mm Pfund) of different floral-type honeys.

mm Pfund							Country	Literature Source
Linden	**Acacia**	**Rapeseed**	**Sunflower**	**Mint**	**Raspberry**	**Chestnut**		
23.1–41.8	0.1–2.0	45.2–73.7	15.5–53.9	18.8–65.2	28.5–43.7	98.3–100.2	Romania	This study
-	-	-	61.3; 70.5; 88.7	-	-	-	Romania	[46]
36.00–54.00	11.00–45.00	-	79.00–83.00	-	-	-	Romania	[47]
-	-	-	32.87–47.52	-	-	-	Romania	[2]
-	-	29.40	37.60	74.30	61.4	-	Romania	[8]
35.64	12.87	36.14	33.66	63.86	63.36	-	Romania	[15]
-	-	-	97.60	-	-	-	Portugal	[36]
22.00–38.00	-	-	39.00–41.00	-	-	-	R. Moldova	[48]
10.00–29.00	1.00–8.00	-	-	-	-	-	Croatia	[45]
68.01; 76.80	-	34.34–114.07	62.07; 114.00	-	87.45; 91.29	-	Poland	[17]
68.1	10.2; 21.47	85.47–114.07	-	-	101.56; 87.45	-	Czech Republic/Poland	[17]
-	26.51	-	-	-	-	-	Germany	[49]
70.00	20.00	-	-	-	-	-	Serbia	[43]
-	-	-	-	-	39–74.5	-	Romania	[50]
-	-	-	-	-	118	-	Spain	[16]
38.27–139.48	−7.26–20.92	2.08–138.56	96.3–198.91	-	-	71.3–149.42	Hungary	[42]
-	-	-	-	-	-	123–150	Spain	[5]
-	-	-	-	-	-	69.63–108.7	Portugal	[51]
33.3	12.9	26.2	52.4	-	-	87.9	Europe	[52]

4.2. Refractive Index, Moisture, and Solid Substances, and Specific Gravity

The main compounds of honey are sugars, and therefore one of its properties is to be hygroscopic. A high moisture content of honey can cause fermentation and spoilage, and these processes negatively change the physicochemical properties and undoubtedly lower the quality and price, shortening the shelf life of the product [9,10,17,21,49,52]. The moisture content of honey samples can be determined by measuring the refractive index. The refractive index of honey is directly related to the content of solid substances. The values of the refractive index (RI) were found between 1.486 and 1.498, with the range of the moisture content between 15.9% for chestnut honey and 20.4% for two samples of linden and rapeseed honey (Table 2). Three honey samples had moisture over the maximum moisture content limit of 20%, recommended for honey in international regulations and Romanian standards [11,37] (20.3% and 20.4% for RP1 and RP2, 20.4% for L3). The various values of moisture content (Table 2) can be attributed to factors such as: weather, harvesting and manipulation procedures, storage, etc. Various values, from 3.9% in acacia honey [53] to 22.8% in acacia honey [54], were also obtained in other studies conducted on the seven honey types analyzed, taken from different countries (Table 10). Honey is a viscous product, having a higher density than water. The specific gravity depends on the moisture content, and it is important to know the stored quantity of honey. The lowest specific gravity of 1.415 g/cm^3 was obtained for linden honey and the highest specific gravity of 1.445 g/cm^3 for chestnut honey. No significant differences were observed between the mean values of all four parameters, neither at the global level (by testing the variability in the dataset of seven honey types) nor between the pairs of honey types (Tables 2 and 5).

Table 10. Comparative physicochemical properties of seven types of monofloral honeys.

Country	Moisture (%)	pH	Free Acidity (meq kg^{-1})	Ash (%)	EC (mS cm^{-1})	TPC mg GAE/100 g	TFC mg QE/100 g	Literature Source
				Linden				
Romania	17.0–20.4	3.81–5.08	23.4–38.6	0.199–0.471	0.358–0.692	22.0–27.5	1.48–2.56	This study
Romania	5.4–6.0	3.6–4.7	-	-	0.410–0.730	-	-	[53]
Romania	16.70–19.10	-	-	0.19–0.30	-	16–38	4.7–6.98	[47]
Romania	16.75	4.05	14.55	-	0.33	-	-	[15]
Romania	17.2–18.8	3.84–4.35	-	-	0.202–0.346	-	-	[54]

Table 10. *Cont.*

Country	Moisture (%)	pH	Free Acidity (meq kg^{-1})	Ash (%)	EC (mS cm^{-1})	TPC mg GAE/100 g	TFC mg QE/100 g	Literature Source
				Linden				
Bulgaria	17.1	4.04	-	-	0.689	-	-	[55]
Croatia	15.9–20.0	-	-	-	0.497–0.628	6.62–12.10	-	[45]
Czech Republic	16	4.06	14.9	-	0.39	45.04	1.88	[13]
Italy	-	-	-	-	-	26	5.5	[56]
Poland	20.30	4.13	25.50	-	0.640	43.69	-	[14]
Poland	17.76	3.81	34.2	-	0.53	38	0.5	[20]
Serbia	15.8; 17.1	4.62; 4.72	14.5; 16.1	-	0.488; 0.608	53.7; 67.3	-	[57]
Serbia	-	-	-	-	-	71.49	-	[43]
Slovakia	18.35	3.90	21.6	-	0.23	35	0.26	[20]
Romania	18.54	4.83	5.88	-	0.512	-	-	[58]
				Acacia				
Romania	17.5–19.5	3.94–4.65	12.8–25.4	0.044–0.190	0.140–0.320	13.7–23.1	0.46–1.29	This study
Romania	3.9–6.2	3.7–4.3	-	-	0.110–0.270	-	-	[53]
Romania	16.60–19.80	-	-	0.03–0.28	-	2.00–39.00	0.91–2.42	[47]
Romania	15.96	4.31	3.86	-	0.12	-	-	[15]
Romania	16.7–22.8	3.65–4.63	-	-	0.097–0.268	-	-	[54]
Bulgaria	16.9	3.23	-	-	0.159	-	-	[55]
Croatia	14.6–19.9	-	-	-	0.1–0.161	2.82–5.20	-	[45]
Czech Republic	17	3.82	9.6	-	0.18	23.84	0.87	[13]
Germany	17	5.4	-	-	-	62.75	-	[59]
Germany	18.83	4.10	-	-	-	21.457	-	[49]
Italy	-	-	-	-	-	18.2	7.6	[60]
Italy	-	-	-	-	-	10.72	3.31	[56]
Poland	17.17	3.77	20.85	-	0.31	14.081	-	[14]
Poland	17.73	3.79	25.6	-	0.42	47	0.32	[20]
Serbia	14.5–18.5	-	6.6–15.5	0.04–0.15	0.083–0.174	58.17–142.61	-	[61]
Serbia	16.4; 17.3	3.90; 4.51	13.8; 16.3	-	0.114; 0.136	13.5; 14.4	-	[57]
Serbia	-	-	-	-	-	37.93	-	[43]
Slovakia	17.86	3.71	16.1	-	0.20	20	0.14	[20]
Turkey	14.45–21.62	-	12–21	-	0.14–0.27	1–3	-	[62]
Croatia	16.78–17.01	-	10.45–11.02	-	0.15–0.18	-	-	[63]
Romania	18.02	4.02	2.29	-	0.218	-	-	[58]
				Rapeseed				
Romania	17.8–20.4	3.95–4.17	24.3–46.6	0.061–0.114	0.209–0.252	22.4–24.1	2.29–2.87	This study
Romania	5.1–5.8	3.6–3.9	-	-	0.150–0.285	-	-	[53]
Romania	18.51	4.23	15.26		0.168	-	-	[58]
Romania	17.31	4.11	17.33	-	0.15	-	-	[15]
Bulgaria	19.7	3.33	-	-	0.181	-	-	[55]
Poland	17.86	3.88	18.6	-	0.23	25	0.32	[20]
Serbia	18.4; 19.4	4.01; 4.10	16.3; 21.3	-	0.191; 0.224	11.5; 11.9	-	[57]
Slovakia	17.45	3.61	13.6	-	0.16	21	0.14	[20]
				Sunflower				
Romania	17.6–19.3	3.91–4.91	35.6–50.1	0.184–0.501	0.469–0.699	21.10–23.90	1.21–2.56	This study
Romania	18.82	4.12	11.82	-	0.367	-	-	[58]
Romania	4.7–6.6	3.3–3.8	-	-	0.340–0.475	-	-	[53]
Romania	18.7	3.656	22.36	0.112	0.301	-	-	[64]
Romania	-	-	-	-	-	48.6–132.5	-	[46]
Romania	17.80–19.70	-	-	0.35–0.40	-	20.00–45.00	11.53–15.33	[47]
Romania	16.23–20.39	3.65–4.34	15.94–47.32	-	0.315–0.441	-	-	[2]
Romania	18.4	3.94	31.6	-	0.362	21.1	22.8	[8]
Romania	16.95	4.04	18.32	-	0.31	-	-	[15]
Romania	17	3.67	-	-	0.188	-	-	[54]
Portugal	19.2	3.84	25.50	0.15	0.235	36.69	1.93	[36]
R Moldova	16.05–17.52	3.68–4.05	-	0.31–0.49	-	-	-	[48]
Serbia	17.4–19.8	-	18.5–39.4	0.12–0.30	0.189–0.359	25.45–61.09	-	[61]
Serbia	17.0	3.38	28.9	-	0.366	27.5	-	[57]
Serbia	14.6–18.6	-	20.40–36.4	0.05–0.30	0.22–0.54	-	-	[65]
Morocco	16.9–18.5	3.52–3.8	15.3–36.7	0.12–0.20	0.43–0.52	-	-	[18]

Table 10. *Cont.*

Country	Moisture (%)	pH	Free Acidity (meq kg^{-1})	Ash (%)	EC (mS cm^{-1})	TPC mg GAE/100 g	TFC mg QE/100 g	Literature Source
Linden								
Mint								
Romania	18.3–18.7	3.99–4.77	38.2–45.7	0.199–0.213	0.503–0.542	50.3–58.7	2.12–3.62	This study
Romania	17.91	4.19	26.71	-	0.466	-	-	[58]
Romania	17.7	4.20	26.9	-	0.474	23.7	25.7	[8]
Romania	16.24	4.52	33.17	-	0.60	-	-	[15]
Tunisia	19.80	-	-	0.13	0.43	119.42	-	[12]
Morocco	15.6–18.3	3.53–4.07	26.6–32	0.18–0.23	0.350–0.505	-	-	[18]
Raspberry								
Romania	17.8–18.7	4.27–4.30	25.8–42.0	0.174–0.176	0.294–0.378	28.2–33.5	2.38–2.87	This study
Romania	17.32–20.12	4.01–4.31	20.1–42.1	-	0.367–0.528	14.48–25.72	25.36–41.35	[50]
Romania	18.3	4.16	27.3	-	0.446	19.9	35.5	[8]
Romania	17.27	4.27	24.06	-	0.519	-	-	[15]
Romania	18.35	4.16	27.31	-	0.439	-	-	[58]
Poland	-	-	-	-	-	109.1	-	[19]
Chestnut								
Romania	15.9–16.0	4.63–4.67	49.6–49.9	0.459–0.502	0.920–0.935	72.5–74.1	4.21–5.27	This study
Spain	15.83	5.31	26.25	-	1.16	35.41	-	[16]
Romania	18.2	5.10	11.2	0.63	1.21	-	-	[66]
Georgia	18.2–20.9	4.5–4.97	20.8–44.6	0.89–2.71	1.036–1.667	80.0–461.5	-	[67]
Spain	-	3.93–4.65	-	-	0.737–1.235	88.43–166.45	6.60–11.78	[5]
Spain	17.6–18.2	4.5–4.7	-	-	1.0–1.1	121.5–138.2	8.4–9.6	[68]
Portugal	15.23–16.87	4.35–4.42	19.67	-	0.98–1.14	67.88–73.39	-	[51]
Turkey	17.4–19.5	4.80–5.34	-	0.74–0.80	1.30–1.52	76.20–94.05	4.20–6.50	[69]

4.3. pH and Free Acidity

Raw honey can be collected at different maturity stages, in different seasons, and its pH ranges between 3.5 and 5.5. The pH values of the analyzed honey samples ranged between 3.81 and 5.08. The concentration of organic acids lowers the pH of honey, giving it an acidic character [10,14,65]. The more acidic the honey is, the more the fermentation process and its alteration are avoided because the medium does not favor the development and growth of microorganisms. The maximum recommended value for free acidity is 50 meq kg^{-1} [11]. In this research, only one sunflower honey sample with 50.1 meq kg^{-1} exceeded the regulated limit. As can be seen from Table 3, the lowest average value of 19.8 meq kg^{-1} for free acidity was determined in acacia honey samples and the highest average value of free acidity was found in the chestnut honey sample (49.8 meq kg^{-1}). Large intervals of free acidity were also reported in other studies, from 2.29 to 47.32 meq kg^{-1} (Table 10). While the pH of the different types of honey did not significantly differ, the free acidity showed a significant difference at the $p < 0.01$ level (Table 3), mainly due to the differentiation of acacia and linden honeys from the other types (Table 5).

4.4. Ash and Electrical Conductivity

The ash content is formed by all the minerals in the honey, an inorganic residue obtained after calcination. In general, blossom honey has an ash content lower than 0.6%, [22,70]. The lowest average mineral content of 0.088% was found in rapeseed samples and the highest average mineral content was found in chestnut honey samples (0.483%). Mărghitaș et al. found the lowest ash content of 0.03% in acacia honey, and in their study, Kharadze et al. reported a 2.1% ash content in chestnut honey (Table 10) [47,67]. Due to its strong correlation with ash, electrical conductivity was included in new quality standards. It is an important parameter, that helps to identify the honey origin, and therefore to differentiate the blossom honey from the honeydew. Electrical conductivity measures inorganic and ionizable organic substances [8,12,17,18,23,65]. The lowest average value of electrical conductivity was found in acacia honey (0.200 mS cm^{-1}) and the highest

average value was for chestnut honey (0.927 mS cm^{-1}). All the analyzed samples complied with the quality criteria of electrical conductivity below 0.8 mS cm^{-1}, the maximum value established by legislation for blossom honey, with the exception of honeydew, chestnut honey, and blends [8,11]. Acacia honey, in most cases, had the lowest amount of ash, and therefore, the electrical conductivity was low. The highest electrical conductivity values for chestnut honey were reported in many studies, within a range between 0.737 and 1.667 mS cm^{-1} (Table 10). The honey samples showed significant differences in terms of ash ($p < 0.01$) and electrical conductivity ($p < 0.001$) when considering the global dataset (Table 3), with the higher differences in conductivity between linden and acacia, and rapeseed and mint, respectively (Table 5).

4.5. Total Phenols Content and Total Flavonoids Content

Honey is known as food and as a natural medicine, used due to its anti-inflammatory, antioxidant, and antibacterial properties. In its composition, there are natural compounds that yield strong antioxidant properties, such as the polyphenolic compounds (phenolic acids, catechins, flavonoids, etc.) [27,71]. These compounds qualitatively and quantitatively varied, and are directly linked with the rich flora, the environment, and the area around the beehive, due to their plant–honey–nectar traceability [13,19,43,44,49,56,72,73]. The chestnut honey sample had the highest average concentration of phenols of 73.2 mg GAE/100 g (Table 3). For the same type of honey from Portugal, similar results in the range of 67.88–73.38 mg GAE/100 g were found by Karabagias et al. [51]. High values of total polyphenols content for chestnut honey from Spain and Georgia ranging from 80.0 to 461.5 mg GAE/100 g were reported by Kharadze et al.; Escuredo et al.; and Rodríguez-Flores et al. [5,67,68]. In acacia honey, total polyphenols were within the range of 13.7–23.1 mg GAE/100 g (Table 3). Similarly, the lowest concentration of total flavonoids (0.87 mg QE/100 g) was determined for acacia honey and the highest content of 4.81 mg QE/100 g in chestnut honey. There was a presence of high amounts of antioxidants in the mint honey of 54.4 mg QE/100 g for polyphenols and 2.87 mg QE/100 g for flavonoids and slightly lower for raspberry honey of 30.9 mg QE/100 g for polyphenols and 2.63 mg QE/100 g for flavonoids. Compared to the results obtained in this study, Pauliuc and Oroian and Pauliuc et al. reported high levels of flavonoids content between 25.36 and 41.35 mg QE/100 g for raspberry honey [8,50].

As summarized in Table 10, studies on antioxidants (TPC, TFC) showed their variable contents in various monofloral honeys. These findings were confirmed by the results of the ANOVA, which indicated significant differences ($p < 0.001$) in the concentrations of both classes of antioxidant compounds in the global dataset (Table 3). For linden honey, TPC ranged between 6.62 and 71.49 mg GAE/100 g, for acacia honey between 1 and 142.61 mg GAE/100 g, for sunflower honey within the range of 20–132.5 mg GAE/100 g, for mint honey between 23.7 and 119.42 mg GAE/100 g, for raspberry honey in the range of 14.48–109.1 mg GAE/100 g, and the highest concentrations were noticed for chestnut honey, between 35.41 and 461.5 mg GAE/100 g. Similarly, different values of TFC were reported: in the range from 0.26 to 6.98 mg QE/100 g for linden honey, from 0.14 to 7.6 mg QE/100 g for acacia honey, and from 1.21 to 22.8 mg QE/100 g for sunflower honey. The highest TFC values were in the range of 2.38 to 41.35 mg QE/100 g in raspberry honey (Table 10). Important factors with a high possibility of influence on the variability in the dataset could be climatic and soil conditions (with large seasonal and yearly variations), as well as the quality of pollen from plants that have different botanical origins and are found in different geographical regions. Different analytical methodologies used to determine these compounds or analytical equipment as possible sources of variation should also be considered [15,51].

However, in all analyzed honey samples, antioxidant compounds were found in variable amounts, which once again proves and confirms the anti-inflammatory, antioxidant, and antibacterial properties of the studied honey samples.

4.6. Mineral Elements' Content

Carbohydrates and water are the main quantitative components of honey, but other important substances such as vitamins and minerals were found in small amounts. Most of the honey components come from plants, but minerals derive from soil following the path of soil–plant–nectar–honey or beehive products [74]. The composition and amount of minerals depend on the environment (soil, water, air), their availability, climatic conditions, botanical origin, and the procedure of harvesting and storage [68,74–76].

The minerals in honeys are highly bioaccessible and play a positive role in human nutrition, prevention of illness, and healing the body. Macro-mineral elements (Ca, K, Na) and trace minerals (Cu, Zn, Mg, Fe) are important in biological systems. Potassium and sodium are electrolytes, and they maintain fluid balance in the body and help the heart and muscle functions. Na also has an essential role in kidney function, the maintenance of optimum blood pressure, and nerve functions. Calcium is involved in mineral homeostasis and physiological performance, has many roles (important for bones, for healing fracture, is indicated in prophylaxis for osteoporosis, and confers a protective role in the musculoskeletal, nervous, and cardiac systems), and acts as a cofactor for several enzymes. Calcium and phosphorus confer a protective role, making honey less cariogenic. Magnesium is a key mineral in honey, which plays an indispensable function in muscle contraction and in transmission of electrical impulses between neurons and contributes to the growth and support of bones and acts as a cofactor for many enzymes, most of which are involved in antioxidant reactions. Mg deficiency contributes to aging and age-related disorders [77–79].

The content of potassium ranged from 46 mg kg^{-1} in rapeseed honey to 1507 mg kg^{-1} in linden honey. The mean concentration of K in the seven studied honey types followed the order: C > L > SF > M > RB > A > RP. The concentrations of calcium were within the range of 58.3–167.5 mg kg^{-1}, following the order: L > C > M > SF > RB > A > RP. The content of magnesium was between 24.8 and 330.6 mg kg^{-1}, the highest in chestnut honey, followed in decreasing order by raspberry honey, sunflower honey, linden honey, mint honey, rapeseed honey, and acacia honey. The lowest amount of sodium (94.5 mg kg^{-1}) was in acacia honey, followed in increasing order by rapeseed honey, raspberry honey, linden honey, mint honey, sunflower honey, and chestnut honey (233.3 mg kg^{-1}). For phosphorus, the lowest average content of 34.1 mg kg^{-1} was found for acacia honey, followed in increasing order by linden honey, rapeseed honey, sunflower honey, mint honey, raspberry honey, and chestnut honey (137.2 mg kg^{-1}) (Table 4). The most abundant element in this study was potassium, and the results are confirmed by several other studies (Table 11). The amount of sodium in the studied honeys was higher compared with the results reported in other studies [55,78,80–83]. The higher concentrations of sodium found in the honey samples collected in this study could be explained by the presence of plants in soils rich in sodium and salts in certain areas in Romania [84]. In the present study, the highest mineral content was determined for potassium, followed by natrium, calcium, and magnesium, and the lowest concentration for phosphorus. Based on the variability in the global dataset, the ANOVA indicated significant differences for Mg and P at the $p < 0.001$ level, and for K at the $p < 0.01$ level (Table 4). No significant differences were observed between the mean values of Ca and Na by testing the variability in the dataset of the seven honey types (Table 4).

In general, the large variations of the studied elements' concentrations were attributed to the composition of the soil, the availability of minerals in the soil, the physiology of the plant, and other factors that can positively or negatively influence the transport of minerals to the nectar. The mineral composition of honey correlates with its color. Dark honeys (such as chestnut honey) contain higher amounts of certain major minerals, such as Ca, K, Mg, and Na, compared with light-colored honeys [79].

Table 11. Mineral elements of the seven floral honeys.

Country	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	P (mg kg^{-1})	Literature Source
			Linden			
Poland	925.2	63.1	28.1	80	-	[75]
Slovenia	1510–2290	48.1–62.5	-	2.9–4.3	-	[80]
Bulgaria	290	46.4	11.5	13.6	-	[85]
Hungary	1027–1883	15.2–67.4	19.8–30.2	5.1–7.4	23.0–42.4	[86]
Croatia	1574.8	387.8	25.5	31.9	-	[87]
Poland	1071.6–2311.6	47.8–102.5	18.9–41.2	-	-	[76]
Romania	955.3	137.9	50.6	123.8	-	[88]
Bulgaria	792	77	21	7.5	49	[55]
Romania	494–735	35.5–76.5	15.7–20.5	22.1–51.1	-	[89]
Hungary	1278	67.9	16.5	9.3	41.5	[81]
Poland	224–528	25.5–48.0	7.3–27	9.2–47.6	35.8–23.8	[82]
			Acacia			
Italy	506	15	5	4.1	-	[90]
Hungary	10–255	17.6–59.6	1.90–15.9	1.60–11.5	27.7–92.3	[86]
Poland	127–196	28.6–69.2	6.5–14	3.8–42.8	71.4–28.6	[82]
Poland	587.2	52.6	24	53.8	-	[75]
Romania	146.7–244.6	1.02–6.9	3.25–6.7	5.06–24.3	-	[74]
Bulgaria	250	46.9	13.1	62.1	-	[85]
Croatia	258.7–360.8	74.4–184.4	16.8–30.7	51.6–168.9	-	[63]
Poland	221.6–431.1	41.7–68.6	16. 7–28.6	-	-	[76]
Romania	553.9	52.9	51.2	171.2	-	[88]
Serbia	188–340	3.8–8.4	3.8–6.8	21–124.3	33–120	[91]
Romania	180.2–252.4	50.1–55.2	11.1–18.1	21.3–24.3	-	[92]
Romania	356–521	5.2–10.2	7.1–18.3	1.9–32.7	-	[89]
Hungary	226.6	12.4	5.2	6.0	24.9	[81]
Bulgaria	126	32	6	8.1	24	[55]
			Rapeseed			
Hungary	103–288	23.7–60.4	13.5–27.6	6.3–16.9	50.9–71.1	[86]
Poland	84.8–494	22.1–62.4	9.5–32.1	7.0–26	35.7–161	[82]
Poland	265.2	48.9	19.2	31.3	-	[75]
Poland	221.6–431.1	41.7–68.6	16.7–28.6	-	-	[76]
Romania	112.6–194.2	87.1–88.6	23.5–23.9	36.1–47.9	-	[92]
Bulgaria	105	46	11	8.5	28	[55]
			Sunflower			
Portugal	276.9	24.9	68.2	87.9	-	[36]
Hungary	245–552	58.2–153	10.2–36.6	4.66–24.5	59.8–144	[86]
Hungary	759	126.4	33.3	13.2	76.3	[81]
Bulgaria	210–260	42.2–56.8	6.9–11	9.5–10.2	-	[85]
Hungary	446.3–790.2	-	24.2–38.7	-	-	[93]
Romania	849.4	163.9	63.8	154.1	-	[88]
Romania	552–574	36.6–60.4	20.4–23.1	24.9–35.2	-	[89]
Romania	234.6–532.1	152.4–200.5	32.6–39.3	47.1–50.7	-	[92]
			Mint			
Tunisia	976.8	221.1	78.1	343.6	59.3	[12]
Spain	200–280	123–148	27.4–36.8	-	-	[18]
Romania	-	1603.5	427.9	-	-	[94]
			Raspberry			
Poland	1104.7	68.8	47.6	48.1	-	[75]
Estonia	125.8–292.7	29.2–53.9	12.1–20.9	4.8–9.7	-	[83]

Table 11. *Cont.*

Country	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	P (mg kg^{-1})	Literature Source
			Chestnut			
Italy	3875	119	49	11.9	-	[90]
Italy	706–714	54–55.9	48.9–49.9	7.5–8.2	143	[82]
Italy	3250–5280	60–130	-	60–90	-	[95]
Slovenia	3670–5520	117–183	-	7.1–9.0	-	[80]
Spain	1615–3770	68–476	30–402	11–84	48–315	[68]
Croatia	2824.4	486.7	59.1	35.8	-	[87]
Hungary	2136–2281	51.6–59.7	25.4–31.7	10.8–18.3	66.4–84.7	[86]
Hungary	1815.8	153	45.4	20.9	79	[81]
Bulgaria	1628	66	16	9.55	32	[55]
Turkey	2524–5125	320.24–463.10	32.05–67.10	28.3–52.0	56.20–71.02	[69]

4.7. FTIR Spectra

The FTIR spectral fingerprints of the seven types of analyzed honeys are shown in Figures 3 and 4. Intense signals were identified in the spectral ranges of 3400–3200 cm^{-1} and 1700–800 cm^{-1}. Many studies carried out on honey have shown that the obtained spectra can be studied by dividing them into spectral regions depending on the vibration of the functional groups:

D0—3500–3100 cm^{-1}, assigned to: O–H stretching (carboxylic acids) and NH_3 stretching (free amino acids).

D1—3000–2800 cm^{-1}, assigned to: C–H stretching (carbohydrates).

D2—1700–1600 cm^{-1}, assigned to: O–H stretching/bending (water), C = O stretching (mainly from carbohydrates), and N–H bending of amide I (mainly proteins).

D3—1540–1175 cm^{-1}, assigned to: O–H stretching/bending, C–O stretching (carbohydrates), C–H stretching (carbohydrates), and C = O stretching of ketones.

D4—1175–940 cm^{-1}, assigned to: C–O, C–C stretching (carbohydrates), and ring vibrations (mainly from carbohydrates).

D5—940–700 cm^{-1}, assigned to: the anomeric region of carbohydrates, C–H bending (mainly from carbohydrates), and ring vibrations (mainly from carbohydrates) [32,33,96,97].

The analyzed honey samples of seven floral origins come from different geographical zones, and the physicochemical analysis results obtained in this study confirmed the differences in FTIR spectra (Table 6). The differences obtained for the FTIR spectra observed in Figure 4 are probably related to the amount of carboxylic acids, groups of polyphenols, types of carbohydrates, or other functional groups.

The spectral region D0 of 3100–3500 cm^{-1} corresponds to O–H of carbohydrates, O–H stretching from water, and N–H stretching vibration (amide A band) of the peptide and proteins and polyphenols [75]. The wavenumber was between 3369 cm^{-1} for chestnut honey and 3416 cm^{-1} for linden honey, higher values compared to those reported by Anjos et al. of 3279.64 cm^{-1}, Sabri and See of 3276.79 cm^{-1}, Pauliuc et al. of 3297 cm^{-1}, or by Svečnjak et al. of 3284 cm^{-1} [30,31,34,58].

In the spectral region between 3000 and 2800 cm^{-1}, the presence of bands between 2922 and 2935 cm^{-1} corresponds to stretching vibrations of the C–H bonds of the chemical structure of the carbohydrates [29,33]. Similar values between 2932 and 2960 cm^{-1} were also reported [31,34,98]. In Figure 4, next to the D1 spectral region, a peak can be observed that records values from 2115 to 2121 cm^{-1}, which can be assigned to C = C conjugated and C ≡ C. Honey spectra recorded peaks between 1636 and 1647 cm^{-1}. It is known that the domain represented by the values from 1700 to 1600 cm^{-1} is responsible for C = O stretching (mainly from carbohydrates), O–H stretching/bending (water), and N–H bending of amide I (mainly proteins). The appearance of peaks could also be attributed to stretching band of carbonyl groups C = O and C = C related to phenolic molecules [29,32,33,58,97]. Comparable wavenumber values were found in studies per-

formed by Anjos et al. of 1646.56 cm^{-1}, Sabri and See of 1638.19 cm^{-1}, Pauluic et al. of 1640 cm^{-1}, and by Svečnjak et al. of 1645 cm^{-1} [30,31,34,58].

In Table 6, the wavenumber values are presented for the spectral region of 1200–1500 cm^{-1}. The bands at 1450 and 1454 cm^{-1} are attributed to bending vibration of O–CH and C–C–H in the carbohydrate structure [31,98].

Characteristic of O–H bending vibration of the C–OH group is the presence of a peak in the spectral range of 1340–1350 cm^{-1}. For 1255–1259 cm^{-1}, the overlapping peaks are due to N–H deformation and C–N stretching vibrations from amide II and C−N amide III bands [31,75,98]. Between 1175 and 940 cm^{-1}, the presence of peaks is associated with C–H in carbohydrates and/or C–O and C–C in carbohydrates [31,34,75,96,98]. The band from 1050 to 970 cm^{-1} is responsible for the C–O stretching vibrations of the C–OH group or for the C–C stretch in the carbohydrate structure, ring vibrations (mainly from carbohydrates) [33,34,98]. The spectral region between 940 and 700 cm^{-1} is assigned to the anomeric region of carbohydrates, C–H bending (mainly from carbohydrates), and ring vibrations (mainly from carbohydrates) specific for honey samples [33,34,75,98].

4.8. Correlation and Multivariate Statistical Analyses

The correlations between the investigated quality parameters (Pearson's coefficients for mean values of each parameter corresponding to each type of honey) are shown in Table 6. Significant correlations at $p < 0.001$ were observed for the refractive index with moisture (r = −0.99), solid substances (r = 0.99), and specific gravity (r = 0.99). These parameters were significantly correlated ($p < 0.01$) with Mg (r = 0.91), P (r = 0.91), and total phenolic content (r = 0.79, $p < 0.05$). Color was significantly correlated ($p < 0.001$) with total flavonoid content (r = 0.95), and with free acidity (r = 0.82), total phenolic content (r = 0.78), and two mineral elements, Mg (r = 0.84) and P (r = 0.86), at the $p < 0.05$ level. Similar correlations have also been reported for other honey samples [36].

As concerns the pH, a strong positive correlation at $p < 0.001$ was observed with K (r = 0.95) and at $p < 0.01$ with Ca (r = 0.93) and Na (r = 0.90), as well as the expected correlations with ash (r = 0.93) and electrical conductivity (r = −0.92).

Significant strong correlations were observed for ash with electrical conductivity (r = 0.97), and all mineral cations (K—r = 0.98, Na—r = 0.95, Ca—r = 0.85, and Mg—r = 0.77). Similar findings were also reported by other authors, who observed strong positive correlation of electrical conductivity with potassium [36,99,100]. Among the mineral elements, P was significantly correlated only with Mg (0.98).

Many studies on honey reported correlations between the studied honey parameters: Strong correlation (Pearson's coefficient of r > 0.8) between color and antioxidant compounds (TPC, TFC) for honey samples from Brazil, Sultanate of Oman, Romania, Bangladesh, Serbia, Croatia, Turkey, and Alger [26–28,43,45,69,101–103]. Positive correlations between color and TPC (Pearson's coefficients of 0.8 > r > 0.6) were found by Kavanagh et al. in Irish honey of r = +0.6 and by Aazza et al. in Portuguese honey of r = +0.685 [36,104]. In this research, the highest content of phenolic compounds was found in chestnut honey, which was the sample with the highest ash content, and at the same time, with a darker color (amber). Rapeseed honey (RP3) of dark color (light amber) also had a high content of ash and antioxidants. The high content of polyphenolic compounds, pollen, pigments (carotenoids and flavonoid), and minerals present in honey can contribute to the appearance of a dark color of the honey [8,49,73]. In this study, comparable values of phenols concentrations in the acacia honey sample (A3) and rapeseed honey sample (RP3) were observed, but the flavonoid content was 3.3 times higher in the RP3 sample compared to sample A3, confirming the conclusions of several studies of a strong positive correlation between color and flavonoid content. Strong correlations (Pearson's coefficient r > 0.8) between total phenol and total flavonoid contents are presented in some studies on honey samples from Italy, Serbia, and Algeria [43,60,103]. Kolayli et al.; in a study on chestnut honey, noticed that the ash content and the value of electrical conductivity increased with the pollen content from the studied chestnut honey samples [69].

Lanjwani and Channa reported good correlations between Na and K ($r > 0.7$) and K and Ca ($r > 0.879$), and moderate correlations ($r = 0.5–0.7$) between Na and Ca and Mg for honey samples from Pakistan [6].

Principal component analysis was used to obtain an overview of the honey data and to achieve a better resolution of contributions from different parameters. Via Varimax normalized factor rotation, three factors that explain 86% of the total variance in the dataset were chosen. The loadings and explained variances are presented in Table 8.

Principal component 1 (PC1) is characterized by the refractive index, moisture, solid substances, and specific gravity as parameters with significant loadings, which can be attributed to some fundamental characteristics of the product that contribute to ensuring its stability. The graphical representation of corresponding scores (Figure 5) indicated that PC1 can be interpreted as a component distinguishing the chestnut honey from the other honey types. PC1 did not clearly differentiate the botanical and geographical origins of the investigated honey types.

PC2, which explains 25.15% of the variance in the dataset, includes pH, ash, electrical conductivity, K, and Na as dominant parameters, suggesting a honey origin factor, as mineral content depends on the botanical and geographical origins of honey [36,74,105]. The scores for PC2 (Figure 5a) did not highlight distinct groupings of the samples according to the geographical origin.

PC3, with high loadings of color and total flavonoid content, followed by free acidity, P, and total phenolic content, could be considered a color/antioxidant factor. The corresponding scores outlined three groups of samples: one group with RP1, RP2, M1, and L2, one group that contained only L1, and one group of all the other samples (Figure 5b).

The hierarchical dendrogram obtained for physicochemical parameters determined in honey samples collected in 2017 is shown in Figure 6. K was clearly differentiated from the other parameters. One cluster contained the mineral elements Na and Ca. Free acidity and total phenols were grouped in a cluster, and electrical conductivity and ash were grouped in another distinct cluster.

5. Conclusions

Some of the investigated quality parameters such as humidity, acidity, and pH are useful when the honey is stored for a longer time, while others (sugar content, electrical conductivity, antioxidant compounds, minerals, etc.) could differentiate the quality of honey for its therapeutic use. FTIR spectroscopy can be easily implemented to determine the composition of honey at mainly a qualitative level and to confirm the authenticity of the honey and its possible adulteration. In all analyzed honey samples, significant amounts of antioxidants were found, that once again proved and confirmed the anti-inflammatory, antioxidant, and antibacterial properties of the studied honey types. The positive correlation between the honey's color and its phenolic and flavonoid content indicates that the higher the content of antioxidant compounds, the darker the honey becomes. The mineral elements' concentrations showed the abundance of potassium and sodium.

Many factors, such as improper management in raising and caring for bees, the presence of xenobiotics (pesticides, heavy metals, etc.), pests, specific diseases of bees, improper food given to bees, and finally, collecting honey from the hive and its subsequent handling, can influence the composition and quality of honey. Hence, every year the quality of honey is different, and therefore a systematic quality control of this product is needed.

Romania has a diverse flora, that allows producing a wide variety of honey types. Most of the samples analyzed in this study were collected from Iasi County, an area in eastern Romania of tradition in linden honey. The great variability of the results on honeys from this relatively small area, especially the mineral elements, in accordance with previous studies carried out on the soils of this area, supports the need of monitoring these honey quality parameters and their continuous updating.

In Romania, and elsewhere, honey is being used as food but also as an adjuvant for various diseases. Today, honey is recommended in different diseases as a dietary supplement. To consume the appropriate dose of honey for each health condition, it is necessary to know the level of antioxidants and the amount of important major elements, such as K, Ca, Na, P, and Mg. Studies shows high differences of these components in the same type of honey. The results reported in this study indicate that studies should not be limited to studying the quality of honey in only one year and from one area because of the very large differences between mineral elements in honey samples collected from very close areas.

In the association of mineral elements' concentration in honey with the particularities of the soil mineral composition, the geographical and floral origins confirm the influence of environmental components (soil, water, air, biota) on the quality of honey. However, for a more complete quality assessment, determination of more mineral elements, including toxic ones, in honey, plants, and soil is still needed.

The obtained data and correlations between the studied parameters of honey are complementary with other studies and highlight the variability of honey composition in time and space, and consequently the need to monitor its quality.

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