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Selected Characteristics of Multifloral Honeys from North-Eastern Romania

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Abstract: The aim of this research was to evaluate some characteristics (moisture, total solid substances, specific gravity, pH, FA, ash, electrical conductivity, TPC and TFC, potassium, calcium, magnesium, sodium, phosphorus, zinc, copper, manganese, nickel, cobalt, and lead) of fifteen multifloral honey samples. The quality of the investigated honey was confirmed by the obtained results: moisture, FA, and EC values were below the limit value regulated by the legislation. The average content of total polyphenols and total flavonoids of 29.91 mg GAE/100 g and 2.13 mg QE/100 g confirm the antioxidant properties of honey. Determination of minerals showed that potassium (101.4–1212.6 mg kg^{-1}) was the most abundant mineral in honey, followed by sodium (40.7–302.3 mg kg^{-1}) and calcium (41.8–230.9 mg kg^{-1}). Lead was found in two samples, with a content under the limit stipulation by legislation; nickel was found in one sample of 0.10 mg kg^{-1}, and the content of cobalt was below the detection limit. Significant correlations ($p < 0.001$) were observed between mm Pfund and electrical conductivity, TPC, TFC, P, Ca, and Zn; strong correlations ($p < 0.001$) were between electrical conductivity with Ash, TPC, TFC, K, and P. FTIR analysis confirmed the differences obtained by analyzing multifloral honey samples.

Keywords: honey; phenols; minerals; FTIR

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1. Introduction

Bees have a special ability to transform the melliferous flower nectar, as well as other sweet secretions present in plants or excretions of various insects, into a sweet product—honey [1–3]. The predominant components in honey are carbohydrates (mainly monosaccharides, such as fructose and glucose) and water. Also, there are many other components in small amounts: enzymes; vitamins (vitamin B6, riboflavin, pantothenic acid); phenolic acids; flavonoids; amino acids; and minerals, all contributing to a unique composition impossible to reproduce [4,5]. Due to its components, honey is known as a complete food, as well as having outstanding therapeutic qualities (antioxidant, antifungal, antibacterial, and antiviral effects) [6,7].

The physico-chemical properties, as well as the organoleptic ones (smell, taste), are greatly influenced by several factors: bee species; seasonal and environmental factors; geographical region. Various activities of the beekeepers can influence the quality of honey. However, the main influence on the overall quality of honey is the floral source [6,8–10]. Due to favorable conditions in terms of climate, as well as the diversity of the melliferous

flora, Romania is an important honey producer [11]. The well-known types of honey produced and marketed in Romania are the common monofloral honeys: acacia; lime; rape; and sunflower. There are regulations established by some European countries regarding the minimum percentage of pollen required for the characterization of monofloral honey [3,12]. Under the limits provided by the legislation, honey is called poly(multi)flora, having as dominant two or more types of different plants' pollen. The properties of the flowers that are mixed together give multifloral honey a special composition (extremely variable), which makes it unique. The melissopalynological analysis is usually used to identify the botanical origin and the pollen spectrum to complete the information on the studied honey samples [13,14]. The studies carried out on this food showed that all these characteristics are closely related [15–17]. European Union Council Directive 2001/110/EC indicates the maximum allowed levels for some parameters, such as 0.1% for the content of water-insoluble solids, 0.5% for pressed honey, 20% moisture content, 50 meq kg^{-1} free acidity, and 0.8 mS cm^{-1} for electrical conductivity for nectar honey (no less than 0.8 mS cm^{-1} for chestnut honey). The acidity of honeys is considered a freshness indicator, considering that at low pH, the growth of microorganisms is inhibited [18–20].

The therapeutic properties of honey are attributed to its antioxidant capacity. The phenolic compounds (flavonoids, phenolic acids) present in honey are responsible for the antioxidant activity, and this sweet food is sometimes used as an ingredient. Flavonoids are floral markers for the geographical and botanical origin of honey and are correlated with some parameters, mainly with total phenol content and color [4,21].

The mineral elements in honey come from the soil and are absorbed by the plants. The uptake from the soil is not selective, and both the essential and toxic to human health minerals (K, Ca, Mg, Na, P, Cu, Mn, Fe) are absorbed (Pb, Cd, Hg). The amount of each element may be an indicator of the environmental quality [22].

The method that is used more and more often due to some advantages (rapidity, nondestructive analytical method) is the Fourier transform infrared spectroscopy (FTIR) technique. This is used to scan and identify substances or chemical groups present in honey, and at the same time, information is received related to quality, the authenticity of the honey, and whether it has been adulterated or not [23,24].

The aim of this research was to characterize multifloral honey samples from North-Eastern Romania from a botanical, physicochemical, and mineral perspective and to find the similarities or differences by using various methods of analysis, including FTIR spectroscopy as a nondestructive method.

2. Materials and Methods

2.1. Honey Samples

Fifteen multifloral honey samples produced by *Apis mellifera* species were collected in October 2017 in Romania. Samples collected from the beekeepers came from two different areas: nine multifloral honey samples (S1, S2, S3, S4, S5, S10, S11, S13, S14) were collected from I-Iasi county (47°15′ N 27°19′ E) and six multifloral honey samples (S6, S7, S8, S9, S12, S15) were collected from II-Vaslui county (46°35′ N 27°46′ E) (Figure 1). Three jars of 400 g for each sample were kept in the dark in a laboratory. Before performing the analyses, the crystallized samples were liquefied at a maximum temperature of 45 °C, and all samples were homogenized.

2.2. Physicochemical Determinations

The botanical origin of the fifteen multifloral honey samples was established using the melissopalynological method of Louvreaux et al. (1978) [25], with some modifications to the centrifugation process (time, speed). Ten grams of sample, dissolved in 20 mL of 5‰ sulphuric acid (Merck KGaA, Darmstadt, Germany), were centrifuged twice (UNIVERSAL 320 HETTICH centrifuge, Hettich GMBH—Tuttlingen, Germany) at 3500 rpm for 30 min. After removing the liquid, 20 mL of distilled water was added and again centrifuged twice at 3500 rpm for 30 min. After removing the liquid, the entire amount of sediment

was placed on a glass slide in two separate drops. After complete drying, the two spots were included in the gelatin–glycerin mixture and covered with lamella. The samples were examined by counting at least 800 pollen grains [26] with an optical microscope (Optika Microscopes Italy, Ponteranica, Italy) under a light microscope with 40× and 100× objective lenses.

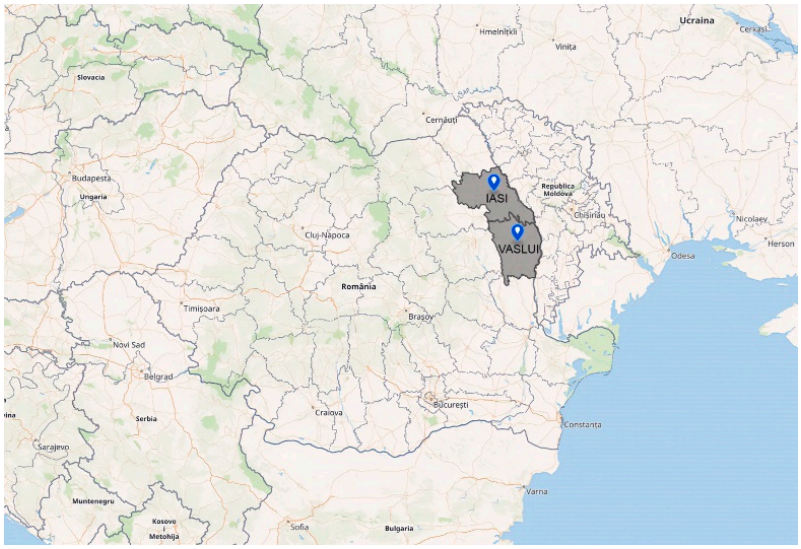


Figure 1. Map showing the areas where the multifloral honey samples were collected.

Fifteen multifloral honey samples were palynological analysed. Relative frequency classes were determined according to the international melissopalyno-logical nomenclature PP—"predominant pollen" (more than 45% of pollen grains counted), SP—"secondary pollen" (representing 15–45% of the total pollen), IMP—"important minor pollen" (3–15%), MP—"minor pollen" (less than 3%) [7].

The Pfund value was determined using the method described by Rațiu et al. [27]. The honey aqueous solutions (50% (*w*/*v*)) were centrifuged (UNIVERSAL 320 HETTICH centrifuge, Hettich GMBH—Tuttlingen, Germany), and the absorbance at 635 nm was measured using a Shimadzu UV-1700 Pharma Spec spectrophotometer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). The absorbance units were converted in mm Pfund using a mathematical relation. The shades of honey color are related to mm Pfund from water white (0 mm Pfund) to dark amber (140 mm Pfund).

By applying the temperature correction, the refractive index was read on an ABBÉ Kruss AR 2008 refractometer (Kruss Scientific GMBH, Hamburg, Germany), and the moisture content (M, %) was determined based on the correspondence between the water content and the refractive index at 20 °C [28].

The total soluble solids (total soluble sugars, Brix degrees) were determined from the correspondence between the refractive index and degrees Brix [29].

Specific gravity was determined by the gravimetric method using a pycnometer device. The results were expressed in g/cm^3 [30].

The pH of the honey solution (10 g of honey in 75 mL of distilled water) was measured using the MULTI 3320 multiparameter (WTW GMBH, Weilheim, Germany) [30].

Free acidity was determined by the titration method: a honey solution (10 g of honey in 75 mL of distilled water) was titrated with 0.1 N NaOH (Chemical Company, Romania), and the result was expressed in meq kg^{-1} [28].

The ash content (g/100 g) was determined by sample calcination in a furnace (Nabertherm B180, Nabertherm GMBH, Lilienthal, Germany) [28].

Electrical conductivity was measured with the MULTI 3320 multiparameter (WTW GMBH, Weilheim, Germany) in a 20% solution (at dry matter) with ultrapure water (Barn-

stead EASY PURE II, Thermo Fisher Scientific Co., Ltd., Des Moines, IA, USA); the electrical conductivity was expressed in mS cm^{-1} [30].

2.3. Total Phenol Content and Total Flavonoid Content

The total phenols and total flavonoids were extracted with an alcoholic solution (1:1 equal parts of methanol (Merck KGaA, Darmstadt, Germany) and acidified water with pH = 2 (adjusted with HCl, Merck KGaA, Darmstadt, Germany), and the extractive honey solution (10%) was homogenized and filtered through filter paper. An aliquot of the 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 Na_2CO_3 (Merck KGaA, Darmstadt, Germany) was added to a total volume of 10 mL. The sample was then incubated for 30 min in the dark at room temperature and spectrophotometrically analyzed at 742 nm. The linear range ($y = 0.0993x + 0.0741$; $R^2 = 0.9991$) for gallic acid (Merck KGaA, Darmstadt, Germany) was 2–12 mg L^{-1}. The total phenol content was expressed as mg of gallic acid equivalents (GAE)/100 g [31,32].

For total flavonoids, equal volumes of 2% $AlCl_3$ (Merck KGaA, Darmstadt, Germany) and the same honey solution used at total phenols determination were mixed, and after 10 min, the absorbance was measured at 430 nm. A standard solution of quercetin (Sigma-Aldrich, St. 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 flavonoid content was expressed as mg of quercetin equivalents (QE)/100 g [31,33].

2.4. Mineral Elements (K, Ca, Mg, Na, P, Zn, Cu, Mn, Ni, Co, and Pb)

The ash resulting from the sample calcination was moistened with ultrapure water and subsequently evaporated, calcinated, treated with 6 M HCl (Merck KGaA, Darmstadt, Germany), heated, and dissolved in 0.1 M nitric acid (Merck KGaA, Darmstadt, Germany). The extract was filtered and diluted with ultrapure water to 25 mL. The phosphorus concentration was spectrophotometrically determined with molybdovanadate reagent (Merck KGaA, Darmstadt, Germany) at 430 nm (Shimadzu UV-1700 Pharma Spec spectrophotometer, Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). The calibration curve was linear in the concentration range of 5–50 mg L^{-1} ($y = 0.0209x + 0.0150$; $R^2 = 1.000$) [34]. Ca, Mg, Zn, Cu, Mn, Ni, Co, and Pb were determined by flame atomic absorption spectrometry. For Ca ($\lambda = 422.7$ nm), the linear range was 1–10 mg L^{-1}, and for Mg Mg ($\lambda = 285.2$ nm) 0.1–0.5 mg L^{-1} (Ca: $y = 0.0264x + 0.0140$, $R^2 = 0.9995$; Mg: $y = 0.3997x + 0.0253$, $R^2 = 0.9991$); the calibration curve for Zn ($\lambda = 213.9$ nm) and Mn ($\lambda = 279.5$ nm) was linear in the concentration range of 0.05–0.6 mg L^{-1} (Zn: $y = 0.4929x + 0.0101$; $R^2 = 0.996$; Mn: $y = 0.0959x + 0.0005$; $R^2 = 0.999$) and for Cu ($\lambda = 324.7$ nm), Ni ($\lambda = 232$ nm), Co ($\lambda = 240.7$ nm), and Pb ($\lambda = 283.3$), the calibration curve was linear in the concentration range of 0.1–1.0 mg L^{-1} (Cu: $y = 0.0097x + 0.2310$; $R^2 = 0.999$; Ni: $y = 0.1036x + 0.0035$; $R^2 = 0.996$ Co: $y = 0.1562x + 0.0054$; $R^2 = 0.997$; Pb: $y = 0.0643x + 0.0031$; $R^2 = 0.997$). Na ($\lambda = 589$ nm; $y = 0.0970x + 0.0017$, $R^2 = 0.996$, 1–10 mg L^{-1}) and K ($\lambda = 766.5$ nm, $y = 0.1010x + 0.0128$, $R^2 = 0.998$, 1–10 mg L^{-1}) were determined by flame atomic emission spectrometry (Analytik Jena novAA 350, Analytik Jena GmbH, Jena, Germany).

2.5. FTIR Spectra

Infrared spectra were obtained using a Jasco FT/IR-660 Plus Fourier Transform Infrared Spectrometer (Tokyo, Japan). A small quantity of liquefied and homogenized samples was incorporated into a KBr (Sigma-Aldrich, Darmstadt, Germany) pellet. Spectral measurements were recorded in the wavenumber range from 4000 cm^{-1} to 400 cm^{-1} (32 scans, resolution 4 cm^{-1}) [23].

2.6. Statistical Analyses

For all the samples, three replicates were analyzed. The results were statistically analyzed (STATISTICA 12.0, StatSoft Inc., Tulsa, OK, USA) to obtain an overview of physicochemical parameter contributions by testing via Pearson's correlation coefficient, principal component analysis, and hierarchical cluster analysis.

3. Results

3.1. Physicochemical Determinations

The plant families of pollen grains identified in the studied honey samples are summarized in Table 1.

Table 1. Plant families of pollen grains in the investigated honey samples.

Family	Sample														
	Area 1									Area 2					
	S1	**S2**	**S3**	**S4**	**S5**	**S10**	**S11**	**S13**	**S14**	**S6**	**S7**	**S8**	**S9**	**S12**	**S15**
Apiaceae	IMP	MP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP
Asteraceae	IMP	IMP	SP	SP	SP	IMP	IMP	SP	IMP	SP	SP	IMP	SP	IMP	SP
Boraginaceae	-	-	-	-	-	-	-	-	-	-	MP	-	MP	-	IMP
Brassicaceae	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
Cyperaceae	-	IMP	IMP	-	-	-	-	-	-	MP	-	MP	-	MP	-
Fabaceae	IMP	SP	IMP	SP	SP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	IMP	SP
Fagaceae	-	MP	MP	-	-	-	-	MP	-	-	-	-	-	-	-
Lamiaceae	MP	-	MP	MP	-	-	MP	-	-	-	MP	-	MP	MP	-
Malvaceae	SP	IMP	IMP	IMP	IMP	MP	IMP	SP	IMP	IMP	IMP	IMP	SP	IMP	IMP
Plantagigaceae	MP	MP	-	-	-	-	MP	MP	-	MP	-	-	-	MP	-
Poaceae	MP	MP	MP	MP	IMP	IMP	IMP	MP	IMP	MP	IMP	IMP	IMP	IMP	MP
Rosaceae	IMP	MP	MP	MP	IMP	SP	SP	-	SP	IMP	-	SP	-	SP	MP
Salicaceae	IMP	IMP	IMP	IMP	IMP	IMP	MP	MP	MP	IMP	SP	IMP	IMP	IMP	IMP

SP—secondary pollen (15–45%); IMP—important minor pollen (3–15%); MP—minor pollen (less than 3%).

Tables 2 and 3 show the results for honey samples from area I and area II, respectively.

Table 2. Physicochemical parameters for multifloral honeys from area I.

Parameter	Descriptive Statistics	Sample								
		S1	**S2**	**S3**	**S4**	**S5**	**S10**	**S11**	**S13**	**S14**
mm Pfund	Min-Max Mean ± SD CV	59.0–60.1 59.4 ± 0.4 0.7	24.1–25.6 24.8 ± 0.5 1.9	55.3–57.1 56.2 ± 0.6 1.0	48.2–50.4 48.9 ± 0.7 1.4	69.0–70.5 69.7 ± 0.5 0.7	40.4–41.9 41.2 ± 0.5 1.3	30.4–31.4 30.9 ± 0.3 1.1	24.4–25.9 25.2 ± 0.5 2.0	44.0–45.6 44.9 ± 0.5 1.1
RI	Min-Max Mean ± SD CV	1.495–1.496 1.496 ± 0.00 0.01	1.493–1.494 1.493 ± 0.00 0.01	1.491–1.492 1.492 ± 0.00 0.01	1.488–1.489 1.488 ± 0.00 0.01	1.488–1.489 1.490 ± 0.00 0.01	1.488–1.489 1.490 ± 0.00 0.01	1.492–1.493 1.492 ± 0.00 0.02	1.491–1.490 1.491 ± 0.00 0.01	1.493–1.494 1.494 ± 0.00 0.01
M %	Min-Max Mean ± SD CV	16.3–16.5 16.4 ± 0.07 0.43	17.4–17.5 17.4 ± 0.04 0.21	17.9–18.0 18.0 ± 0.04 0.22	19.2–19.4 19.3 ± 0.06 0.29	19.1–19.2 19.1 ± 0.05 0.28	19.0–19.2 19.2 ± 0.06 0.33	17.5–17.8 17.6 ± 0.09 0.51	18.0–18.2 18.1 ± 0.05 0.28	17.0–17.2 17.1 ± 0.05 0.29
TSS °Brix	Min-Max Mean ± SD CV	82.0–82.2 82.1 ± 0.07 0.08	81.0–81.1 81.0 ± 0.03 0.04	80.5–80.6 80.6 ± 0.04 0.05	79.1–79.3 79.2 ± 0.06 0.07	79.4–79.5 79.4 ± 0.05 0.07	79.3–79.5 79.4 ± 0.06 0.08	80.7–81.0 80.9 ± 0.09 0.11	80.2–80.5 80.4 ± 0.05 0.06	81.3–81.4 81.4 ± 0.06 0.07
SG g/cm^3	Min-Max Mean ± SD CV	1.440–1.442 1.441 ± 0.00 0.03	1.434–1.435 1.434 ± 0.00 0.01	1.431–1.432 1.431 ± 0.00 0.02	1.421–1.422 1.421 ± 0.01 0.03	1.423–1.424 1.423 ± 0.00 0.03	1.422–1.424 1.423 ± 0.00 0.03	1.432–1.434 1.433 ± 0.00 0.04	1.429–1.430 1.430 ± 0.00 0.02	1.436–1.437 1.436 ± 0.00 0.03

RI—refractive index; M—moisture; TSS—total soluble solids; SG—specific gravity; SD—standard deviation; CV—coefficient of variation.

Table 3. Physicochemical parameters for multifloral honeys from area II.

Parameter	Descriptive Statistics	Sample					
		S6	**S7**	**S8**	**S9**	**S12**	**S15**
mm Pfund	Min-Max	46.3–48.2	25.2–26.3	63.4–65.3	42.3–43.4	47.6–48.6	13.8–15.0
	Mean ± SD	47.5 ± 0.64	25.8 ± 0.42	64.5 ± 0.53	42.9 ± 0.33	48.0 ± 0.34	14.5 ± 0.41
	CV	1.36	1.63	0.81	0.77	0.71	2.87
RI	Min-Max	1.494–1.495	1.494–1.495	1.491–1.492	1.487–1.488	1.494–1.495	1.493–1.494
	Mean ± SD	1.495 ± 0.00	1.495 ± 0.00	1.492 ± 0.00	1.488 ± 0.00	1.495 ± 0.00	1.494 ± 0.00
	CV	0.01	0.01	0.01	0.02	0.02	0.01
M %	Min-Max	16.6–16.8	16.8–16.9	17.9–18.0	19.4–19.6	16.6–16.9	17.0–17.1
	Mean ± SD	16.7 ± 0.06	16.8 ± 0.05	18.0 ± 0.06	19.5 ± 0.09	16.7 ± 0.09	17.1 ± 0.03
	CV	0.38	0.29	0.33	0.46	0.56	0.20
TSS °Brix	Min-Max	81.7–81.9	81.6–81.7	80.5–80.7	79.0–79.2	81.6–81.9	81.4–81.5
	Mean ± SD	81.8 ± 0.06	81.7 ± 0.04	80.6 ± 0.06	79.0 ± 0.09	81.8 ± 0.09	81.4 ± 0.03
	CV	0.07	0.05	0.07	0.11	0.11	0.04
SG g/cm^3	Min-Max	1.438–1.440	1.438–1.439	1.431–1.432	1.420–1.421	1.438–1.440	1.436–1.437
	Mean ± SD	1.439 ± 0.00	1.438 ± 0.00	1.431 ± 0.00	1.420 ± 0.00	1.423 ± 0.00	1.436 ± 0.00
	CV	0.03	0.02	0.03	0.04	0.05	0.02

RI—refractive index; M—moisture; TSS—total soluble solids; SG—specific gravity, SD—standard deviation; CV—coefficient of variation.

Table 4 summarizes the analysis results for pH, free acidity, ash, electrical conductivity, total phenol content, and total flavonoid content of honey samples from area I.

Table 4. pH, free acidity, ash, electrical conductivity, total phenol content, and total flavonoid content of multifloral honeys from area I.

Parameter	Descriptive Statistics	Sample								
		S1	**S2**	**S3**	**S4**	**S5**	**S10**	**S11**	**S13**	**S14**
pH	Min-Max	4.26–4.27	5.01–5.02	3.73–3.74	4.24–4.25	3.85–3.86	3.78–3.79	3.58–3.59	4.07–4.08	3.75–3.76
	Mean ± SD	4.26 ± 0.0	5.02 ± 0.00	3.74 ± 0.0	4.25 ± 0.0	3.86 ± 0.0	3.79 ± 0.0	3.58 ± 0.0	4.08 ± 0.0	3.76 ± 0.0
	CV	0.03	0.02	0.02	0.02	0.03	0.08	0.09	0.07	0.07
FA meq kg^{-1}	Min-Max	47.9–48.3	28.5–28.8	39.9–40.5	38.5–39.0	49.4–50.6	34.3–34.9	43.2–43.9	42.4–42.9	23.7–24.4
	Mean ± SD	48.1 ± 0.14	28.7 ± 0.10	40.3 ± 0.19	38.8 ± 0.16	49.9 ± 0.40	34.7 ± 0.18	43.6 ± 0.22	42.7 ± 0.17	24.1 ± 0.21
	CV	0.29	0.35	0.46	0.42	0.80	0.52	0.50	0.41	0.89
Ash %	Min-Max	0.211–0.300	0.436–0.515	0.201–0.281	0.125–0.191	0.258–0.384	0.128–0.144	0.092–0.105	0.224–0.314	0.160–0.185
	Mean ± SD	0.233 ± 0.03	0.484 ± 0.03	0.241 ± 0.03	0.155 ± 0.02	0.325 ± 0.05	0.133 ± 0.01	0.099 ± 0.00	0.280 ± 0.03	0.176 ± 0.01
	CV	11.60	5.36	11.49	13.99	13.97	6.08	4.20	9.59	4.48
EC mS cm^{-1}	Min-Max	0.537–0.539	0.735–0.736	0.295–0.297	0.399–0.401	0.503–0.504	0.302–0.305	0.318–0.319	0.498–0.499	0.496–0.497
	Mean ± SD	0.538 ± 0.00	0.736 ± 0.00	0.296 ± 0.00	0.400 ± 0.00	0.504 ± 0.00	0.304 ± 0.00	0.318 ± 0.00	0.499 ± 0.00	0.496 ± 0.00
	CV	0.16	0.10	0.24	0.21	0.10	0.29	0.17	0.11	0.11
TPC mg GAE/100g	Min-Max	34.43–35.44	28.06–29.26	30.91–32.56	23.66–24.37	38.83–40.34	30.86–31.67	32.58–33.41	28.40–29.84	32.89–34.87
	Mean ± SD	34.86 ± 0.35	28.63 ± 0.38	31.73 ± 0.71	24.05 ± 0.26	39.54 ± 0.47	31.23 ± 0.27	33.03 ± 0.31	29.02 ± 0.47	33.93 ± 0.70
	CV	1.00	1.33	2.22	1.08	1.20	0.86	0.95	1.62	2.05
TFC mg QE/100g	Min-Max	2.38–2.85	1.69–2.04	2.26–2.74	1.99–2.35	2.60–3.01	1.67–2.17	1.75–2.10	1.64–1.97	2.18–2.78
	Mean ± SD	2.63 ± 0.16	1.92 ± 0.11	2.51 ± 0.16	2.18 ± 0.13	2.75 ± 0.13	1.97 ± 0.16	1.97 ± 0.11	1.77 ± 0.10	2.41 ± 0.18
	CV	5.98	5.86	6.29	6.06	4.56	7.92	5.75	5.74	7.47

FA—free acidity; EC—electrical conductivity; TPC—total phenol content; TFC—total flavonoid content; SD—standard deviation; CV—coefficient of variation.

Table 5 shows the results of pH, free acidity, ash, electrical conductivity, total phenol content, and total flavonoid content of honey samples from area II.

3.2. Mineral Elements (K, Ca, Mg, Na, P, Zn, Cu, Mn, Ni, Co, and Pb)

Table 6 shows the content of macroelements and microelements determined in multifloral honey from area I.

Table 7 shows the content of macroelements and microelements determined in multifloral honeys from area II.

Table 5. pH, free acidity, ash, electrical conductivity, total phenol content, and total flavonoid content of multifloral honeys from area II.

Parameter	Descriptive Statistics	Sample					
		S6	**S7**	**S8**	**S9**	**S12**	**S15**
pH	Min-Max	3.84–3.85	3.94–3.95	4.04–4.05	3.77–3.78	3.88–3.89	3.92–3.93
	Mean ± SD	3.84 ± 0.00	3.95 ± 0.00	4.05 ± 0.00	3.78 ± 0.00	3.88 ± 0.00	3.92 ± 0.00
	CV	0.03	0.02	0.02	0.07	0.09	0.03
FA meq kg^{-1}	Min-Max	49.4–50.0	47.6–48.0	41.5–41.7	20.7–21.4	46.3–47.1	18.9–19.4
	Mean ± SD	49.7 ± 0.21	47.8 ± 0.14	41.6 ± 0.09	21.0 ± 0.22	46.8 ± 0.23	19.1 ± 0.19
	CV	0.42	0.29	0.21	1.07	0.50	0.10
Ash %	Min-Max	0.177–0.261	0.062–0.089	0.190–0.214	0.111–0.132	0.146–0.158	0.061–0.078
	Mean ± SD	0.216 ± 0.03	0.079 ± 0.01	0.201 ± 0.01	0.121 ± 0.01	0.152 ± 0.00	0.070 ± 0.01
	CV	12.98	11.74	4.02	5.09	2.76	7.46
EC mS cm^{-1}	Min-Max	0.485–0.486	0.208–0.209	0.510–0.511	0.300–0.302	0.418–0.419	0.168–0.170
	Mean ± SD	0.486 ± 0.00	0.209 ± 0.00	0.511 ± 0.00	0.301 ± 0.00	0.419 ± 0.00	0.169 ± 0.00
	CV	0.10	0.21	0.10	0.24	0.13	0.42
TPC mg GAE/100g	Min-Max	26.37–27.78	25.73–26.73	27.96–29.07	29.88–31.41	26.67–27.84	22.78–23.65
	Mean ± SD	26.88 ± 0.0.48	26.22 ± 0.34	28.65 ± 0.35	30.43 ± 0.73	27.24 ± 0.42	23.18 ± 0.31
	CV	1.77	1.31	1.21	2.38	1.53	1.33
TFC mg QE/100g	Min-Max	2.27–2.84	1.08–1.61	1.87–2.11	1.94–2.34	2.05–2.44	1.36–1.85
	Mean ± SD	2.57 ± 0.19	1.28 ± 0.15	2.00 ± 0.08	2.11 ± 0.12	2.28 ± 0.13	1.60 ± 0.16
	CV	7.26	11.72	4.00	5.68	5.60	10.08

FA—free acidity; EC—electrical conductivity; TPC—total phenol content; TFC—total flavonoid content; SD—standard deviation; CV—coefficient of variation.

Table 6. Macroelement and microelement content (mg kg^{-1}) of multifloral honeys from area I.

Parameter	Descriptive Statistics	Sample								
		S1	**S2**	**S3**	**S4**	**S5**	**S10**	**S11**	**S13**	**S14**
K	Mean ± SD	818.6 ± 4.06	1212.6 ± 18.68	110.6 ± 1.63	112.3 ± 1.76	403.9 ± 2.07	210.1 ± 2.09	354.1 ± 1.50	486.9 ± 2.99	415.7 ± 2.58
	CV	0.50	1.54	1.48	1.56	0.51	1.00	0.42	0.61	0.62
Ca	Mean ± SD	116.1 ± 2.70	88.9 ± 3.52	141.2 ± 1.27	199.9 ± 1.39	46.5 ± 1.93	99.4 ± 1.97	102.5 ± 1.39	164.4 ± 2.14	195.3 ± 1.92
	CV	2.33	3.96	0.90	0.70	4.15	1.98	1.36	1.30	0.99
Mg	Mean ± SD	64.1 ± 2.85	43.6 ± 1.73	40.3 ± 1.83	44.9 ± 1.55	61.2 ± 1.55	40.0 ± 1.54	35.8 ± 1.83	56.9 ± 1.89	58.7 ± 1.31
	CV	4.44	3.97	4.56	3.44	2.53	3.86	5.11	3.33	2.22
Na	Mean ± SD	144.5 ± 3.61	279.8 ± 5.39	113.2 ± 1.46	302.3 ± 1.71	139.6 ± 1.73	221.7 ± 1.59	112.1 ± 1.71	75.3 ± 1.78	100.7 ± 1.98
	CV	2.50	1.93	1.29	0.57	1.24	0.72	1.53	2.36	1.97
P	Mean ± SD	61.9 ± 1.39	54.6 ± 1.28	46.9 ± 0.87	31.8 ± 1.45	85.5 ± 1.83	51.3 ± 2.06	42.8 ± 1.17	44.3 ± 1.94	58.3 ± 1.83
	CV	2.25	2.34	1.86	4.55	2.14	4.02	2.72	4.39	3.13
Zn	Mean ± SD	1.33 ± 0.03	2.87 ± 0.03	4.85 ± 0.03	6.19 ± 0.18	5.11 ± 0.10	1.58 ± 0.08	0.76 ± 0.07	5.69 ± 0.09	4.66 ± 0.07
	CV	2.10	1.22	0.71	2.83	2.02	5.01	9.23	1.58	1.53
Cu	Mean ± SD	1.01 ± 0.01	0.96 ± 0.02	1.08 ± 0.04	1.30 ± 0.09	1.12 ± 0.11	1.82 ± 0.07	1.49 ± 0.08	1.99 ± 0.08	1.61 ± 0.08
	CV	1.42	2.33	3.97	6.61	9.58	4.09	5.66	4.18	5.23
Mn	Mean ± SD	0.43 ± 0.01	0.77 ± 0.02	0.27 ± 0.02	0.53 ± 0.04	4.31 ± 0.15	1.17 ± 0.09	0.43 ± 0.03	1.62 ± 0.0	0.51 ± 0.04
	CV	3.17	3.16	7.52	7.48	3.51	7.67	8.09	3.39	7.83
Ni	Mean ± SD									
	CV	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Co	Mean ± SD									
	CV	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pb	Mean ± SD									
	CV	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

SD—standard deviation; CV—coefficient of variation; LOD—limit of detection.

Table 7. Macroelement and microelement content (mg kg^{-1}) of multifloral honeys from area II.

Parameter	Descriptive Statistics	Sample					
		S6	**S7**	**S8**	**S9**	**S12**	**S15**
K	Mean ± SD CV	471.5 ± 1.89 0.40	150.0 ± 1.76 1.17	639.8 ± 1.64 0.26	224.5 ± 2.12 0.95	424.4 ± 4.74 1.12	101.4 ± 1.83 1.81
Ca	Mean ± SD CV	225.5 ± 1.58 0.70	92.5 ± 0.89 0.96	230.9 ± 1.93 0.84	78.7 ± 1.96 2.49	120.6 ± 2.68 2.22	41.8 ± 1.37 3.29
Mg	Mean ± SD CV	65.5 ± 1.91 2.92	60.5 ± 1.77 2.92	56.6 ± 1.23 2.18	37.7 ± 1.43 3.79	48.4 ± 1.54 3.18	32.7 ± 1.45 4.43
Na	Mean ± SD CV	94.8 ± 2.12 2.24	40.7 ± 1.91 4.70	165.2 ± 1.44 0.87	241.1 ± 2.12 0.88	178.6 ± 1.12 0.63	84.6 ± 1.86 2.20
P	Mean ± SD CV	63.9 ± 2.40 3.75	36.5 ± 1.13 3.10	67.3 ± 1.66 2.47	39.4 ± 1.25 3.16	60.8 ± 1.88 3.10	28.6 ± 1.62 5.66
Zn	Mean ± SD CV	7.23 ± 0.16 2.21	6.18 ± 0.14 2.30	13.66 ± 0.18 1.32	4.44 ± 0.10 2.23	1.54 ± 0.08 5.49	4.97 ± 0.10 1.96
Cu	Mean ± SD CV	2.18 ± 0.13 5.99	0.77 ± 0.07 8.95	1.99 ± 0.08 4.05	2.12 ± 0.09 4.38	0.76 ± 0.07 8.80	1.49 ± 0.04 2.93
Zn	Mean ± SD CV	7.23 ± 0.16 2.21	6.18 ± 0.14 2.30	13.66 ± 0.18 1.32	4.44 ± 0.10 2.23	1.54 ± 0.08 5.49	4.97 ± 0.10 1.96
Cu	Mean ± SD CV	2.18 ± 0.13 5.99	0.77 ± 0.07 8.95	1.99 ± 0.08 4.05	2.12 ± 0.09 4.38	0.76 ± 0.07 8.80	1.49 ± 0.04 2.93
Ni	Mean ± SD CV	<LOD	0.10 ± 0.01 12.04	<LOD	<LOD	<LOD	<LOD
Co	Mean ± SD CV	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pb	Mean ± SD CV	<LOD	0.05 ± 0.01 14.29	<LOD	0.01 ± 0.00 5.17	<LOD	<LOD

SD—standard deviation; CV—coefficient of variation; LOD—limit of detection.

3.3. FTIR Spectra

In Table 8, the main maximum absorption wavelengths of honey sample spectra are presented. Figures 2 and 3 show the matrix plot of multifloral honey in the 4000–400 cm^{-1} range and the scores of the first two principal components computed by Principal Component Analysis, respectively.

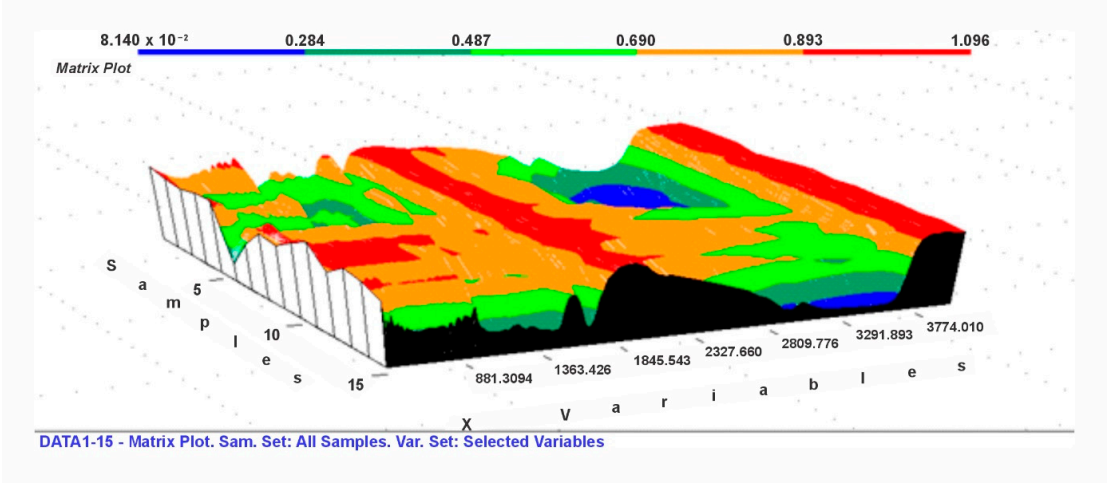


Figure 2. Matrix plot of multifloral honey in 4000–400 cm^{-1} domain.

Table 8. The position of the main bands obtained from FTIR multifloral honey samples analysis.

Sample	Spectral domains				
	D1 3500–3100 cm^{-1}	D2 3000–2800 cm^{-1}	D3 1700–1600 cm^{-1}	D4 1540–1175 cm^{-1}	D5 1175–940 cm^{-1}
	assigned to O–H stretching (carboxylic acids) and NH3 stretching (free amino acids).	assigned to: C–H stretching (carbohydrates).	C=O stretching (mainly from carbohydrates) and N–H bending of amide I (mainly proteins). O–H stretching/bending (water) and N–H bending of amide I (mainly proteins), C=C	assigned to O–H stretching/bending, C–O stretching (carbohydrates), C–H stretching (carbohydrates), and C=O stretching of ketones	assigned to C–O, C–C stretching (carbohydrates), and ring vibrations (mainly from carbohydrates).
1	3414	2931; 2882	1643	1538; 1416; 1384, 1257	1055; 919
2	3392	2932; 2878	1644	1538, 1415; 1384, 1257	1054; 920
3	3396	2932, 2878	1644	1416; 1257; 1143	1056; 918
4	3409	2933; 2884	1644;	1418; 1257; 1142	1057; 918
5	3413	2934; 2865	1644	1416; 1359, 1256, 1149,	1057; 918
6	3375	2935; 2878	1641	1413; 1346; 1253; 1140	1056; 920
7	3440	2939; 2868	1645	1553; 1452; 1251;1148	1011; 918
8	3417	2936; 2876	1644	1451; 1414; 1250, 1149	1014; 920
9	3440	2921; 2853	1645	1555; 1453; 1239; 1143	1007; 926
10	3444	2920; 2870	1645	1531; 1453; 1227; 1146	1012; 925
11	3442	2923; 2865	1645	1554; 1453; 1234; 1165	1012; 924
12	3440	2925; 2854	1645	1554; 1452; 1385, 1140	1032; 919
13	3417	2925; 2859	1645	1555; 1454; 1241; 1136	1031; 978; 916
14	3415	2930; 2872	1645	1538; 1453, 1245, 1146	1051; 922
15	3504	2934; 2878	1647	1415; 1259; 1146	1083; 919

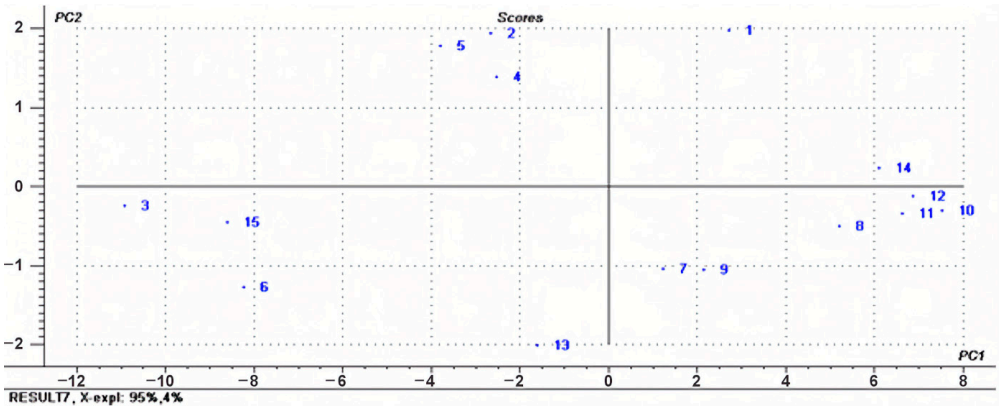


Figure 3. Principal Component Analysis.

3.4. Correlation and Multivariate Statistical Analysis

Table 9 summarizes the correlations between all the determined characteristic parameters of the analyzed honey samples.

Table 9. Pearson's correlation coefficients between the investigated honey parameters (significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)).

	mm Pfund	RI	M	TSS	EC	SG	pH	FA	Ash	TPC	TFC	K	Ca	Mg	Na	P	Zn	Cu	Mn
mm Pfund	1.00																		
RI	**−0.21** *	1.00																	
M	**0.23** **	**−1.00** ***	1.00																
TSS	**−0.21** *	**1.00** ***	**−1.00** ***	1.00															
EC	**0.30** ***	0.11	−0.12	0.12	1.00														
SG	**−0.21** *	**1.00** ***	**−1.00** ***	**1.00** ***	0.13	1.00													
pH	**−0.17** *	0.11	−0.12	0.11	**0.67**	0.11	1.00												
FA	**0.45** ***	**0.23** **	**−0.22** *	**0.23** **	**0.18** *	**0.23** **	−0.09	1.00											
Ash	**0.20** *	−0.00	−0.01	0.01	**0.85** ***	0.02	**0.70** ***	0.14	1.00										

Table 9. *Cont.*

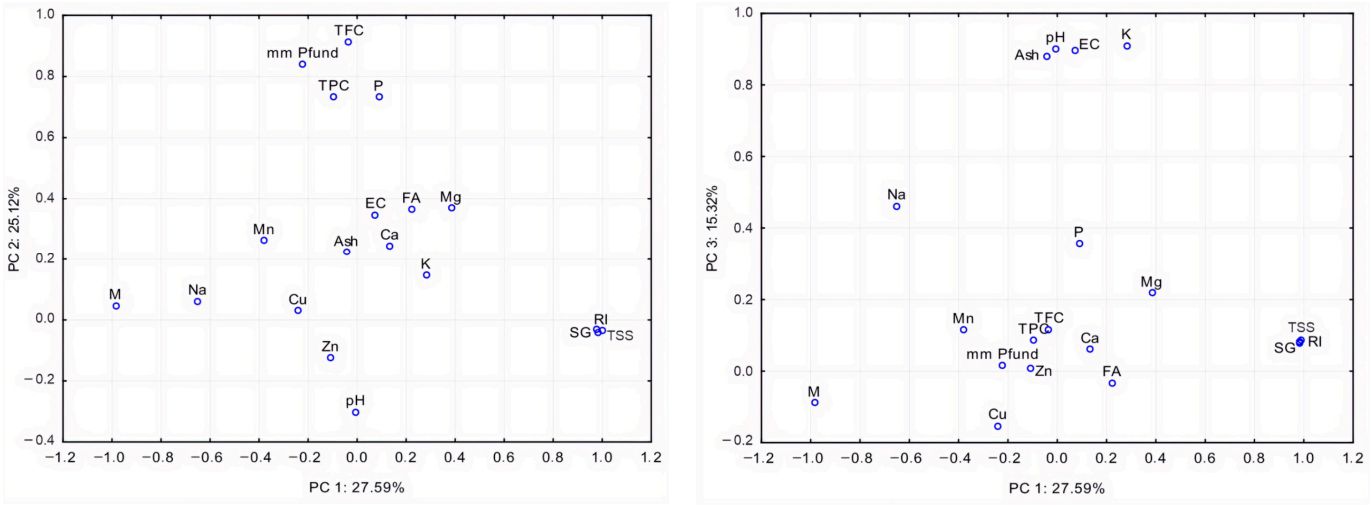
	mm Pfund	RI	M	TSS	EC	SG	pH	FA	Ash	TPC	TFC	K	Ca	Mg	Na	P	Zn	Cu	Mn
TPC	**0.55** ***	**−0.18** *	**0.17** *	**−0.17** *	**0.30** ***	−0.17	**−0.22** **	**0.23** **	**0.34** ***	1.00									
TFC	**0.75** ***	−0.05	0.05	−0.05	**0.40** ***	−0.04	−0.13	**0.27** ***	**0.35** ***	**0.57** ***	1.00								
K	0.07	**0.33** ***	**−0.34** ***	**0.34** ***	**0.89** ***	**0.34** ***	**0.74** ***	0.10	**0.78** ***	**0.22** **	0.17	1.00							
Ca	**0.33** ***	0.12	−0.12	0.12	**0.33** ***	0.13	−0.01	**0.23** **	**0.03**	**−0.21** *	**0.22** *	0.09	1.0000						
Mg	**0.43** ***	**0.37** ***	**−0.36** ***	**0.36** ***	**0.46** ***	**0.36** ***	0.09	**0.60** ***	**0.29** ***	**0.28** ***	**0.33** ***	**0.32** ***	**0.42** ***	1.00					
Na	**0.18** *	**−0.55** ***	**0.55** ***	**−0.55** ***	**0.33** ***	**−0.56** ***	**0.50** ***	**−0.28** ***	**0.28** ***	−0.09	0.14	**0.25** **	0.00	**−0.36** ***	1.00				
P	**0.71** ***	0.08	−0.07	0.08	**0.61** ***	0.09	0.02	**0.46** ***	**0.51** ***	**0.65** ***	**0.64** ***	**0.48** ***	**0.14** *	**0.61** ***	−0.03	1.00			
Zn	**0.27** **	−0.10	0.11	−0.10	0.07	−0.09	0.02	0.06	−0.01	**−0.28**	−0.10	−0.05	**0.56** ***	**0.34** ***	−0.13	0.14	1.00		
Cu	−0.01	**−0.33** ***	**0.33** ***	**−0.33** ***	−0.03	**−0.33** ***	**−0.31** ***	**−0.27** ***	**−0.18** *	−0.07	0.01	−0.12	**0.41** ***	−0.01	−0.02	−0.02	**0.40** ***	1.00	
Mn	**0.33** ***	**−0.40** ***	**0.41** ***	**−0.40** ***	0.21 *	−0.41 ***	−0.08	**0.28** ***	**0.34** ***	**0.51** ***	**0.30** ***	0.02	**−0.30** ***	**0.35** ***	−0.07	**0.55** ***	0.12	0.10	1.00

The values of significant correlation coefficients are marked in bold.

The extracted principal components and the corresponding eigenvalue are shown in Table 10 and Figure 4. Figure 5 shows the hierarchical dendrogram obtained by cluster analysis.

Table 10. Loadings and corresponding variance (%) for the extracted principal components for the analyzed honey samples.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
mm Pfund	−0.22	0.84	0.02	0.29	0.03	0.27
RI	0.99	−0.04	0.08	0.00	−0.06	0.10
M	−0.99	0.05	−0.09	0.01	0.07	−0.09
TSS	0.99	−0.04	0.08	0.00	−0.07	0.09
EC	0.08	0.35	0.89	0.17	0.06	−0.04
SG	0.99	−0.04	0.09	0.00	−0.06	0.09
pH	−0.01	−0.30	0.90	0.00	−0.16	0.19
FA	0.22	0.37	−0.03	0.25	0.29	0.68
Ash	−0.04	0.22	0.88	−0.05	0.20	0.05
TPC	−0.10	0.73	0.09	−0.34	0.43	−0.08
TFC	−0.03	0.91	0.12	0.00	−0.05	0.01
K	0.28	0.15	0.91	−0.02	0.03	−0.07
Ca	0.13	0.25	0.06	0.85	−0.31	−0.08
Mg	0.38	0.37	0.22	0.49	0.44	0.26
Na	−0.65	0.06	0.46	−0.09	−0.52	0.05
P	0.09	0.73	0.36	0.13	0.44	0.09
Zn	−0.11	−0.12	0.01	0.87	0.18	−0.01
Cu	−0.24	0.03	−0.15	0.50	0.11	−0.74
Mn	−0.38	0.26	0.12	−0.03	0.83	0.09
Eigenvalue	5.24	4.77	2.91	2.17	1.22	1.01
% Total variance	27.59	25.12	15.32	11.40	6.41	5.30
Cumulative %	27.59	52.71	68.03	79.43	85.84	91.14



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

Figure 4. Graphical representation of scores for (**a**) PC2 versus PC1 and (**b**) PC3 versus PC1 for the analyzed honey samples.

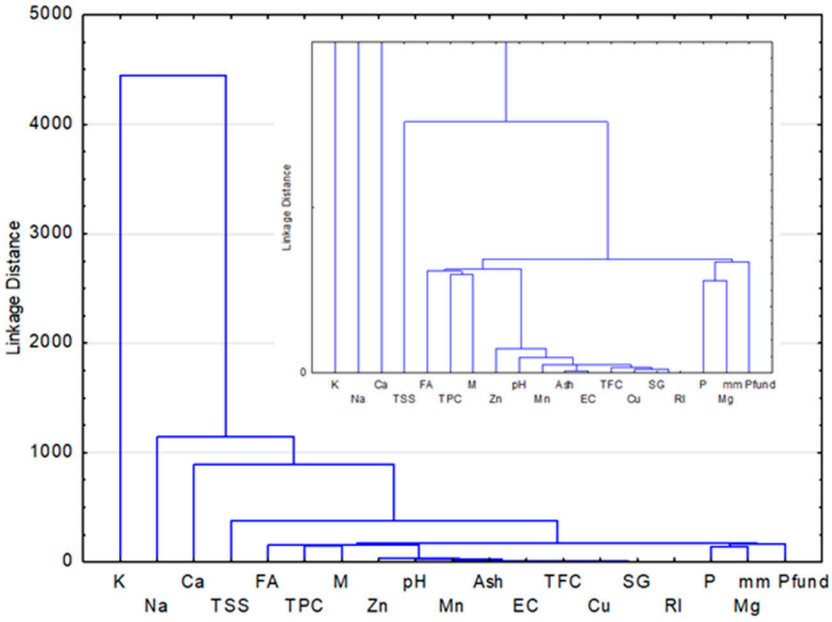


Figure 5. Hierarchical dendrogram obtained by cluster analysis.

4. Discussion

4.1. Physicochemical Determinations

Melissopalynological analysis showed that all honey samples are multifloral. The pollen grain types identified in the honey samples were part of families with various species: *Apiaceae* (*Angelica* sp., *Eryngium* sp.); *Asteraceae* (*Achillea* sp., *Taraxacum* sp., *Helianthus* sp., *Marticaria chamomilla*, *Centaureae* sp.); *Boraginaceae* (*Symphytum* sp.); *Brassicaceae* (*Brassica* sp.); *Cyperaceae* (*Carex* sp.); *Fabaceae* (*Robinia* sp., *Trifolium* sp., *Vicia* sp.m, *Medicago* sp.); *Fagaceae* (*Quercus* sp., *Fagus* sp.); *Lamiaceae* (*Mentha* sp., *Salvia* sp.); *Malvaceae* (*Tillia* sp.); *Plantagigaceae* (*Plantago* sp.); *Poaceae* (*Festuca* sp., *Sorghum* sp., *Zea mays*); *Rosaceae* (*Crataegus* sp., *Pyrus* sp., *Prunus* sp.); *Salicaceae* (*Populus* sp., *Salix* sp.) (Table 1). Secondary pollen (16–45%) was from the following families: *Asteraceae* (S3, S4, S5, S13 from Area 1 and S6, S7, S9, S15 from Area 2); *Brassicaceae* (in all samples); *Fabaceae* (S2, S4, S5 from Area 1 and S15 from Area 2); *Malvaceae* (S1, S13 from Area 1 and S9 from Area 2); *Rosaceae* (S10, S11, S14 from Area 1 and S8, S12 from Area 2). The main pollen in the analyzed samples was from

the *Brassicaceae* family (19.8–35.8%); the pollen grains from other families varied: *Apiaceae* (2.3–14.3%); *Asteraceae* (5.5–33.7%); *Boraginaceae* (2.0–3.1%); *Cyperaceae* (2.3–3.0%); *Fabaceae* (3.7–32.3%); *Fagaceae* (1.0–2.1%); *Laminaceae* (0.5–2%); *Malvaceae* (2.8–29.7%); *Plantagigaceae* (0.5–2.2%); *Poaceae* (0.5–7.2%); *Rosaceae* (1.0–31.9%); *Salicaceae* (1.0–16.8%). There are no criteria for the definition of monofloral honey. Some countries have established national conditions for the amount of pollen in one flower to classify it as monofloral. Minimum percent of pollen for the characterization of monofloral honey is excepted in *Robinia pseudoacacia* (Italy—15%; Germany—20%; Croatia—20%; Serbia—20%; Romania—25%), *Tilia* spp. (Germany—20%; Croatia—25%; Serbia—25%; Romania—30%), and *Helianthus annuus* (Romania—40%) [3,12]. The differences can be seen in the FTIR spectral analysis, where, after examining sample S1, linden honey (*Tillia* sp. = 29.7%) could also be considered monofloral, according to the regulations of other countries. In Table 1, the similarity of plant species in both areas of origin of the analyzed honey samples can be observed.

The color of honey is influenced by various factors, such as water content, HMF content, phenolic compounds, carotenoids, the amount or type of mineral elements, pollen floral types, geographical origin, time, and technological conditions (temperature, processing/handling/storage), etc. [5,33,35–37]. Honey color ranges from nearly colorless to dark brown; thus, the color of honey was classified into seven categories: water white, extra white, white, extra light amber, light amber, amber, and dark amber [32]. The color of multifloral honey samples varied from 14.5 mm Pfund (white) to 69.7 mm Pfund (light amber) in both areas I and II (Tables 2 and 3). Much research found different colors of the same type of honey: color varied from 0.1 mm Pfund in Romanian acacia honey [38] to 20.0 mm Pfund in Serbian acacia honey [35]; Bodor et al. 2021 [36] noted large differences in the color of the Hungarian samples within the same botanical group (linden), from 38.27 mm Pfund to 139.48 mm Pfund. For monofloral honey, in general, it is known that acacia honey is light-colored and chestnut honey is dark-colored; for polyflora honey, the color is given by a large number of pollen types.

Like other food, honey has water in its composition. Shelf life for foods is important because consumers should know when food is safe to be consumed. Increasing the water content can generate the fermentation process and spoilage in honey, raising its susceptibility to microbes; the physico-chemical properties, taste, texture, and aspects of a product change negatively. [22,32,39,40]. The moisture content of multifloral honey samples from area I ranged between 16.4% and 19.3%, and for samples from area II ranged between 16.7% and 19.5% (Tables 2 and 3). These values are below 20%, the maximum limit recommended for honey stipulated in Romanian standards and international regulations [28,41]. Moisture content values under this limit were found in many multifloral honey samples: from 13.91% to 15.80% in honey from Portugal [22]; from 17.11% to 17.93% in multifloral honey from Poland [42]; from 17.4% to 18.4% in multifloral honey from Chile [10]; and from 15.9 to 19.6% [13,37,43] in Romanian multifloral honey. It is known that honey is hygroscopic, and its water content is influenced by factors such as floral origin, geographical location, climatic conditions, level of maturity, the harvest season, and the water from the honey must be constantly checked [16].

The total soluble solids in honey are mainly sugars. Most honey samples had values higher than 80 Brix° of total soluble solids. When the percent of total soluble solids increases, the percent of moisture decreases, and honey has better stability during storage. According to the grading system of the United States Department of Agriculture, when results exceed 80 Brix° (<20% water), honey is qualitative [29]. Four multifloral honey samples (S4, S5, S9, S10) have lower values, from 79.0 Brix° to 79.4 Brix° (Tables 2 and 3).

Honey is heavy, with a mean value of specific gravity of 1.4 g/cm^3. Specific gravity has a practical significance in keeping track of the amount of honey safely stored because it is correlated with moisture content. The mean values of the specific gravity of multifloral honey samples ranged between 1.420 g/cm^3 and 1.441 g/cm^3 (Tables 2 and 3).

Honey has an acidic pH due to the presence of different organic acids (acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic, and succinic). Organic acids are respon-

sible for the flavor and aroma and are important for honey preservation. The lower value of pH in honey inhibits the growth of microorganisms. The majority of honey samples have pH values between 3.5 and 5.5 [13,42,44]. In our study, the mean values of pH were in the 3.58–5.02 range. The values of free acidity obtained in both studied areas ranged between 19.1 meq kg^{-1} and 49.9 meq kg^{-1} (Tables 4 and 5). Similar values were obtained for Romanian honey [13,37,44] and honey from Poland [42,45]. Two samples (S5-area I and S6-area II) with free acidity of 49.9 meq kg^{-1} and 49.7 meq kg^{-1}, respectively, had values close to 50 milliequivalents acid per 1000 g, the maximum allowed value specified by the legislation [Council Directive 2001/110/CE 2002], and honey must be periodically checked if is stored for a longer period.

The average values of ash content were found between 0.070% for the S15 multifloral honey sample and 0.48% for the S2 multifloral honey sample. The amount of all minerals in blossom honey is lower than 0.6; the ash content is variable due to factors such as atmospheric conditions, mineral content in the soil, and the physiology of the plant [16,17]. Due to its strong correlation with ash, the results were easily obtained, and electrical conductivity was included in new standards. The 0.8 mS cm^{-1} is the maximum value established by legislation for blossom honey [41]. Several investigations on honey samples showed similar values to the ones obtained in the present study [42,44–46]. The electrical conductivity showed variable values from 169 mS cm^{-1} (S15) to 736 mS cm^{-1} (S2) (Tables 4 and 5). Analyzing the maximum and minimum values of the ash content with the electrical conductivity values of the multifloral honey samples S2 and S15, a positive correlation can be observed between the two parameters. Ash content and electrical conductivity are parameters that can indicate the botanical origin of bee honey, whether it is blossom honey or not.

From ancient times, honey was known to have therapeutic properties (antioxidant, antibacterial, bacteriostatic). The antioxidants that occur in honey are phenolic acids and flavonoids; quantitatively, the amount of these compounds can largely vary, with a close relation with the type of plant (phenolic compounds are secondary metabolites of plants) and the environment quality traceability [8,18]. The total phenol values of multifloral honey analyzed were found to be between 23.18 mg GAE/100 g and 39.54 mg GAE/100 g. The minimum flavonoid content obtained was 1.28 mg QE/100 g for the S7 sample, and the maximum value for the S5 sample was 39.54 mg QE/100 g (Tables 4 and 5). The average content of total polyphenols and total flavonoids of 29.91 mg GAE/100 g and 2.13 mg QE/100 g confirm the antioxidant properties of multifloral honey samples. High values of total phenol content in the multifloral honey from the Czech Republic were obtained by Halouzka et al., 2016, from 36.3 mg GAE/100 g to 72.3 mg GAE/100 g, and total flavonoid content was 3.54 mg QE/100 g [18]. Multifloral analyzed honey samples from Azerbaijan honey also had a high content of polyphenols, between 18.824 GAE/100 g and 87.350 GAE/100 g [47].

A study on multifloral honey from Northern Romania showed lower values of TPC than the results in this study, between 6.28 mg GAE/100 g and 12.94 mg GAE/100 g [43]. Studies carried out on multifloral honey highlight the influence of the type of plant, region, and quality of the environment on total phenol content: values ranging between 23.69–102.16 mg GAE/100 g were found on multifloral honey from Poland [45]; lower results were obtained by Bertoncelj et al., 2007, of 12.68–19.46 mgGAE/100 g on Slovenian multifloral honey [8]. The results of TPC and TFC in our research are lower compared to the results of total phenol content (350.80–565.90 mg GAE/100 g) and of total flavonoid content (29.01–29.48 mg QE/100 g) of multifloral honey from Banat Region of Romania found by Pătruică et al., 2022 [2]; also, increased values were obtained in the study conducted by Giosanu et al., 2022, on multifloral honey samples from the south of Romania (80.19–170.79 mg GAE/100 g and 3.13–19.64 mg QE/100 g) [15].

4.2. Mineral Elements (K, Ca, Mg, Na, P, Zn, Cu, Mn, Ni, Co, and Pb)

The amount of ash in honey depends on the quality of the environment, the quality of the soil, the physiology of the plants, the climate, etc. Following the well-known soil–plant–nectar–pollen–honey path, it is not only the amount of minerals that is important but also the type of elements with which honey is enriched. There are many minerals in honey, such as macroelements (calcium, potassium, magnesium, sodium) and microelements (iron, manganese, copper, zinc, nickel, lead, and cadmium). Some elements have an important role in the human organism; some other elements are toxic. Potassium, calcium, sodium, magnesium, and phosphorus are the main honey macroelements; microelements such as zinc, copper, manganese, and nickel are present in honey in small amounts and are essential for the normal function of the human body and regulate many biological functions [10,48–50]. The content of potassium ranges from 101.4 mg kg^{-1} in the S15 honey sample to 1212.6 mg kg^{-1} in the S2 honey sample. Similar values were obtained for Romanian honey by Tudoreanu et al., 2012, and Barbeș et al., 2021 [48,50]. The elements obtained in this study ranged between 41.8 mg kg^{-1} and 230.9 mg kg^{-1} for Ca, 32.7 mg kg^{-1} and 65.5 mg kg^{-1} for Mg, 40.7 mg kg^{-1} and 302.3 mg kg^{-1} for Na, 28.6 mg kg^{-1} and 85.5 mg kg^{-1} for P, 0.76 mg kg^{-1} and 13.66 mg kg^{-1} for Zn, 0.76 mg kg^{-1} and 2.18 mg kg^{-1} for Cu, and between 0.27 mg kg^{-1} and 4.31 mg kg^{-1} for Mn (Tables 6 and 7). The higher concentrations of potassium and sodium found in the multifloral honey samples from area I could be explained by the presence of plants on soils rich in potassium, sodium, and salts in Iasi County [51].

In this study, the concentration of some elements was determined: cobalt and nickel are elements that occur naturally in small quantities in the environment but can cause negative health effects (allergenic potential, lung inflammation). Lead is a toxic heavy metal with no physiological role in the human body [46,52]. In all multifloral honey samples, cobalt was below the limit of detection. The maximum limit for Pb in honey is 0.1 mg kg^{-1}, which was established by the European Commission [EU 2023/915]. Values below the legal limits of 0.01 mg kg^{-1} and 0.05 mg kg^{-1} were recorded for Pb in the S9 and S7 multifloral samples, and in one sample (S7), Ni of 0.1 mg kg^{-1} was found. The absence or very low content of toxic elements below the limit of detection indicates that the sources of honey were not contaminated.

Many studies have been performed related to the presence of mineral elements in honey samples collected from polluted and intensively industrialized areas, as well as honey collected from unpolluted areas (Zn values were between 0.004 mg kg^{-1} and 36.40 mg kg^{-1}; Cu values were between LOD and 33.00 mg kg^{-1}, and Pb content ranged was between LOD and 3.41 mg kg^{-1}). The results lead to the same conclusion: all elements of the environment (water, air, soil) positively or negatively influence the quality of the product [2,10,13,42,46,48–50,53–59].

4.3. FTIR Spectra

Much research on honey has shown that the obtained spectra can be studied by dividing them into band domains, depending on the vibration of the functional groups [60,61]. The FTIR spectra for the analyzed honey samples show a number of common characteristics but also a number of differences. In the 4000–3500 cm^{-1} range, a series of sharp bands specific to O–H valence vibrations corresponds to O–H of carbohydrates, and O–H stretching (carboxylic acids) appears. In the D1 range, 3500–3100 cm^{-1}, a broad band appears, specific to water molecules (O–H stretching from water) in the samples, but also some small shoulders/inflections that can be attributed to N–H stretching vibration (amide A band) of the peptides and proteins and polyphenols. D2 domain range, 3000–2800 cm^{-1}, is assigned to C–H stretching (carbohydrates), symmetric and antisymmetric. The asymmetric band appears around 2930 cm^{-1}, while the symmetric band is much weaker and appears around 2870 cm^{-1} (the presence of bands between 2940 and 2850 cm^{-1} corresponds to asymmetric and symmetric stretching vibrations of the C–H bonds of the chemical structure of the carbohydrates). Bands between 2200 cm^{-1} and 2100 cm^{-1} can be assigned to C=C conjugated

and C≡C. In the D3 domain, 1700–1600 cm^{-1}, an intense band appears, centered around 1645 cm^{-1}, specific to C=O stretching (mainly from carbohydrates) valence vibrations. In the same interval, a series of weaker bands specific to O–H stretching/bending vibrations (water), N–H bending of amide I (mainly proteins), and C=C related to phenolic molecules can appear. D4, 1540–1175 cm^{-1}, is assigned to O–H stretching/bending, C–O stretching (carbohydrates), C–H stretching (carbohydrates), and C=O stretching of ketones. The bands at 1450 cm^{-1} and 1454 cm^{-1} correspond to the bending vibration of the O–CH and C–C–H bonds of the carbohydrates. The peak in the spectral range of 1340–1350 cm^{-1} and 1255–1259 cm^{-1} is characteristic of the O–H bending vibration of the C–OH group. The peaks corresponding to N–H deformation and C–N stretching vibrations from amide II and C–N amide III bands overlap. The peaks within the range 1165 cm^{-1}–1136 cm^{-1} correspond to C–H in carbohydrates and/or C–O and C–C in carbohydrates. D5, 1100–900 cm^{-1} is assigned to C–O, C–C stretching (carbohydrates), and ring vibrations (mainly from carbohydrates). The range between 900 cm^{-1} and 600 cm^{-1} is assigned to the anomeric part of carbohydrates, C–H bending (from carbohydrates), and ring vibrations (from carbohydrates) specific to honey. The main bands for the analyzed samples are presented in Table 8. The principal component analysis, PC1, PC2, presented in Figure 3 in the range 4000–400 cm^{-1}, indicates that the samples are different from each other, but similarities may appear between some samples. The distribution on the dials shows the changes that occur in the fingerprint characteristic field. In addition, it can be observed that sample S1 is totally different from the other samples. Samples S2, S4, and S5 are similar as are samples S3, S6, S15 and S7, S8, S9, S10, S11, S12, and S14. Sample S13 shows characteristics between the last two groups of samples. The spectral domains that contribute to the differentiation of honey samples can also be seen very well from the Matrix plot shown in Figure 2 (domain 4000–400 cm^{-1}). Results from other studies showed the variability of honey compounds: the characteristic peaks obtained by Mail et al., 2019 were as follows: 3272 cm^{-1}; 2934 cm^{-1}; 1643 cm^{-1}; 1416 cm^{-1}; 1345 cm^{-1}; 1256 cm^{-1}; and 1026 cm^{-1}; the characteristic peaks obtained by Aykas, 2023 were as follows: 3285 cm^{-1}; 2930 cm^{-1}; 1637 cm^{-1}; 1411 cm^{-1}; 1321 cm^{-1}; 1254 cm^{-1}; 1110 cm^{-1}; 1043 cm^{-1}; and 918 cm^{-1}; the characteristic peaks obtained by Giosanu et al., 2022 were as follows: 3233 cm^{-1}; 2935 cm^{-1}; 1646.9 cm^{-1}; 1418 cm^{-1}; 1338 cm^{-1}; 1247 cm^{-1}; 1151 cm^{-1}; 1043 cm^{-1}; and 918 cm^{-1} [15,24,62].

4.4. Correlation and Multivariate Statistical Analysis

Pearson correlation coefficients between honey parameters and the extracted PC are shown in Tables 9 and 10. A moderate correlation was observed between TPC and TFC (0.57) and between mmPfund and FA, TPC, and Mg (0.45, 0.55, 0.43), and a strong correlation (r = 0.75) was found for mmPfund with TFC and for mmPfund with P (r = 0.71). Strong correlations ($p < 0.001$) are between EC with Ash (r = 0.85), TPC, and K (r = 0.89) with P (r = 0.61). There are positive moderate correlations between the following minerals: P with Mg (r = 0.61); P with K (r = 0.48); Ca with Zn (r = 0.56); and P with Mn (r = 0.55). The research on the correlations between the quality parameters of honey has reported similar correlations both between different types of honey and within the same type of honey. Lanjwani et al., 2019, reported a good correlation between macrominerals Na, K, Mg, and Ca for honey samples from Pakistan [49]; the correlation between the color of honey and the content of mineral salts was reported by Karabagias et al., 2014 [63]; the correlation between the color of honey and the antioxidant compounds was found by Bertoncelj et al., 2007 [8]. Similar low correlations between TPC and TFC were found by Uçar et al., 2023 (−0.3418) in multifloral honey samples from Northern Cyprus, Sant'Ana et al., 2014 (0.5) in Brazilian honey samples from Northern Cyprus, and Cabrera et al., 2017 (0.45) in Argentinian honey samples [64–66]. Low values of Pearson coefficients of correlation between mmPfund and antioxidant compounds (TPC) were also reported for Algerian honey (0.693), for Irish honey (0.6), for honey from Brazil (0.4), and a correlation of 0.53 for Argentinian honey samples [65–68]. A strong correlation of 0.711 was found between color and TPC by Daci-Ajvazi et al., 2017 for multifloral honey from Kosovo [69]. Lower values of Pearson

coefficients of correlation between mmPfund and antioxidant compounds (TFC) of 0.6 and 0.685 were observed for Brazilian and Australian honeys, respectively [65,70]. Similar values of Pearson coefficients to those found in this research (0.78) were also obtained by Cabrera et al., 2017, from Argentinian honey samples and Živković et al., 2019 (0.771) from honey samples from Serbia [35,66].

The hierarchical dendrogram obtained for physicochemical parameters determined in multifloral honey samples collected in 2017 is shown in Figure 5 and indicates that K is clearly differentiated from the other parameters, similar to Na and Ca. One cluster contains the mineral elements P and Mg. Cu and SG are grouped in a cluster, and electrical conductivity and ash are grouped in a distinct cluster.

5. Conclusions

Melissopalynological analysis confirms the multifloral characteristics of the studied samples of honey.

All the quality indicators determined in this study were within the limits stipulated by the legislation. The multifloral honey samples have antioxidant potential through the amount of phenols and flavonoids, and the presence of these antioxidants confirms its therapeutic character.

Determination of minerals showed that potassium is the most abundant mineral in honey, followed by calcium and sodium. The high values of K and Na reflect the amount of these minerals in the soil. The presence of macro and microminerals ensures honey has a place in the food list.

The limited number of samples with Pb content over the detection limit indicates the existence of few possible pollution sources. The lead content value was below the limit recommended by legislation.

The use of the FTIR spectral method confirms the difference between the investigated samples by analyzing the pollen and could also highlight the differences in the chemical composition of honey.

This study confirms the close connection between the composition of honey and the quality of the environmental elements.

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