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Assessment of Total Phenolic and Total Flavonoid Contents and Their Correlation with Some Physicochemical Parameters of Monofloral Romanian Honey †

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Abstract: The purpose of this study was to assess the total phenolic and flavonoid contents from 28 samples Romanian raw monofloral honey (acacia; linden; rapeseed, sunflower and mint) and to establish their correlations with several qualitative parameters. Pearson test shown strong positive correlation of total phenols content with total flavonoids (r = 0.76) and color intensity (r = 0.72). For total flavonoids content, correlations were: strong positive with color intensity (r = 0.81), ash content (r = 0.76) and electrical conductivity (r = 0.73). The relevant levels of polyphenols and flavonoids identified in the analyzed honey demonstrate its antioxidant potential, as essential nutritional and sanogenic features in human nutrition.

Keywords: honey; quality; phenolic content; flavonoid content; Pearson's correlation

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1. Introduction

Romanian beekeeping sector was developed throughout the latest few years and conventional and organic honey production has been increase [1]. Honey bee has been used as food and also as medicine since ancient times. The chemical composition consists mainly of sugars, about 80%, mainly glucose and fructose, 15–17% water, 0.1–0.4% protein and 0.2% ash, while other components contained in small quantities give some special properties [2]. The antimicrobial, anti-inflammatory, antioxidative activity are properties that have been recognized their beneficial effects on the human body. Many studies have shown that the composition and antioxidant activity of honey depends on several factors that can directly or indirectly affect its quality such as: bee species, geographic area, plants, weather, harvesting technique and storage conditions [3,4]. Antioxidant capacity of this food has been correlated with the amount of some substances that are present in honey composition: enzymes, polyphenolic compounds (phenolic acids, phenolic acid derivates, flavonoids), proteins, amino acids and other compounds. Flavonoids belong to a larger group of plant phenolic compounds. These bioactive molecules, present in plants, are brought together with the collected nectar and they have been used as floral markers for identify the geographical and botanical origin of honey. [5–7]. Literature reports correlations between physico-chemical parameters: ash with electrical conductivity, total phenols content with color and total flavonoids content [8–11]. The aim of this research was to assess the total phenolic and total flavonoids contents and to and to establish their correlations with several qualitative parameters.

2. Materials and Methods

Twenty-eight samples of raw monofloral honey types: acacia (A)-eight samples, linden (L)-seven samples, rapessed (R)-five samples, sunflower (SF)-five samples and mint (M)-three samples were collected in 2019 directly from beekeepers. The honey bee samples issued from Eastern and South-Eastern Romanian sites of Iasi county (A1, A2, A3, A4, L1, L2, R1, R2, SF1, SF2), Vaslui county (A5, A6, L3, L4, L5, R3, R4, SF3), Botosani county (A7, A8, L6, L7, R5, SF4, SF5) and Tulcea county (M1, M2, M3). The raw honey samples were kept at 20 ± 3 °C in the dark. The preparing stage of raw honey samples, for the analysis, consisted in liquefying the crystallized samples at 40 °C in a water bath (manufacturer: Memmert GMBH, Schwabach, Germany). They were subsequently homogenized and filtered through a gauze.

The color of honey samples was measured using the Shimadzu UV-1700 Pharma Spec instrument (manufacturer: Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). Honey aqueous solutions with 50% (*w*/*v*), were centrifuged at 3200 rpm for 5 min at UNIVERSAL 320 HETTICH centrifuge (manufacturer: Hettich GMBH, Tuttlingen, Germany) [12]. The absorbance units, measured at 635 nm wavelength, were converted in mm Pfund with the relation:

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

where Pfund = the honey color value in the Pfund scale [mm]; Abs = the value of the absorbance read at the wavelength of 635 nm.

Ten grams of honey sample weighed on the PI-214 DENVER analytical balance (manufacturer: Denver Instrument GMBH, Gottingen, Germany) was dissolved in distilled water, filtered through filter paper with constant weight and washes several times. After dried in ESAC 100 oven at 105 °C (manufacturer: SC Electronic April Aparatura Electronica Speciala S.R.L -Cluj-Napoca, Romania), the content of water insoluble solids was calculated by the difference between filter paper with water insoluble solids weight and filter paper weight and the result was expressed as a percentage [13].

The refractive index (RI) of samples was read on the ABBÉ Kruss AR 2008 refractometer (manufacturer: Kruss Scientific GMBH, Hamburg, Germany) and moisture content (M) was taken from the table of correspondence between the water content and the refractive index at 20 °C [13]. Solid substances content (SS), expressed as a percentage, was calculated as the difference between 100 and moisture content.

The total soluble solids (TSS) represented by soluble sugars, expressed as Brix degrees, were read from the table of correspondence between the refractive index at 20 °C and degrees Brix [14.

Specific gravity was obtained by gravimetric method, using the pycnometer device. The results were expressed in g/cm^3 [15].

Using the MULTI 3320 multiparameter (manufacturer: WTW GMBH, Weilheim, Germany), the pH values were measured in a honey solution (10 g of honey in 75 mL of distilled water). On the same solution free acidity was found by titration with 0.1 N NaOH solution using phenolphthalein as a color indicator. [13,16,17].

Assessment of ash content was carried out by gravimetric method after honey samples were calcinated in the Nabertherm B180 furnace (manufacturer: Nabertherm GMBH, Lilienthal, Germany); the results were expressed in g/100 g. Electrical conductivity was measured with the MULTI 3320 multiparameter (manufacturer: WTW GMBH, Weilheim, Germany). The 20% solution (the weighted honey was calculated at dry matter) was made with ultrapure water produced by the Barnstead EASY PURE II system (manufacturer: Thermo Fisher Scientific co. ltd., IA, USA) and the results were expressed in μS cm^{-1} [13,16].

Total phenols content and total flavonoids content were determined according to the method Folin-Ciocalteu with minor modification. A 10% honey alcoholic solution (methanol/acidified water-dionised water, pH = 2, HCl) was homogenized and filtered through

filter paper. An aliquot of honey solution was mixed with 0.2 mL of Folin-Ciocalteu reagent for 5 min and added of 75 g/L Na_2CO_3 until volume was 10 mL. Solution was measured at 742 nm against a blank at the Shimadzu UV-1700 Pharma Spec instrument (manufacturer: Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) after keep in the dark at room temperature for 30 min. To obtain the calibration curve in 5 calibration points (concentration ranging in 2–12 mg L^{-1} interval; $y = 0.089x + 0.1147$; $R^2 = 0.9972$) it was used as standard gallic acid. The maximum absorption was recorded at 742 nm for a spectrum range of 700–800 nm. The results were expressed in mg of gallic acid equivalents (GAE)/100 g [17,18].

For determination of total flavonoids content, the same honey alcoholic solution antimicrobial, anti-inflamatory, antioxidative activity. The same volume of 2% $AlCl_3$ was added to this honey solution. After 10 min, absorbance was read at 430 nm wavelength. To obtain the calibration curve in 6 calibration points (concentration ranging in 0.5–5 mg L^{-1} interval; $y = 0.1331x + 0.0112$; $R^2 = 0.9997$) it was used as standard quercetin. The maximum absorption was recorded at 430 nm for a spectrum range of 400–500 nm. The results were expressed in mg of quercetin equivalents (QE)/100 g [8,18].

The analytical data were processed statistically with SPSS Statistical version 26.0. Correlations were tested by using Pearson's correlation coefficient (r) between total phenols and total flavonoids content and the others investigated parameters. Analyses were made in triplicate.

3. Results

3.1. Physicochemical Analyses

The results (color, water insoluble solids, refractive index, moisture, solid substances content, total soluble solids, specific gravity) are presented in Table 1.

Table 1. Parameters (color, water insoluble solids, refractive index, moisture, solid substances, total soluble solids and specific gravity) of honey samples (n = 28).

Type	Descriptive Statistics	Color mm Pfund	WIS %	RI	M %	SS %	TSS %	SG %
Acacia	Min–Max	0.2–7.5	0.035–0.108	1.488–1.498	15.41–19.49	80.51–84.59	79.03–83.06	1.420–1.448
	Mean ± SD	3.9 ± 2.29	0.079 ± 0.03	1.494 ± 0.00	16.98 ± 1.21	83.02 ± 1.21	81.51 ± 1.20	1.437 ± 0.01
	CV	71.20	33.81	0.21	7.15	1.46	1.47	0.58
Linden	Min–Max	21.7–26.7	0.062–0.107	1.488–1.493	17.28–19.20	80.80–82.72	79.32–81.19	1.422–1.435
	Mean ± SD	24.5 ± 1.75	0.090 ± 0.01	1.491 ± 0.00	18.10 ± 0.70	81.90 ± 0.70	80.40 ± 0.68	1.430 ± 0.00
	CV	7.14	16.19	0.12	3.87	0.86	0.85	0.33
Rapeseed	Min–Max	52.5–61.0	0.074–0.107	1.486–1.495	16.77–20.07	79.93–83.23	78.28–81.73	1.410–1.439
	Mean ± SD	55.6 ± 3.29	0.095 ± 0.01	1.491 ± 0.00	18.21 ± 1.35	81.79 ± 1.35	80.27 ± 1.39	1.428 ± 0.01
	CV	5.92	13.42	0.23	7.40	1.65	1.74	0.80
Sunflower	Min–Max	36.9–82.9	0.060–0.114	1.487–1.494	16.93–19.60	80.40–83.07	78.92–81.58	1.420–1.438
	Mean ± SD	61.5 ± 18.92	0.080 ± 0.02	1.491 ± 0.00	18.27 ± 1.23	81.73 ± 1.23	80.23 ± 1.22	1.429 ± 0.01
	CV	30.77	26.87	0.21	6.76	1.51	1.53	0.59
Mint	Min–Max	42.8–86.1	0.047–0.087	1.489–1.496	16.07–18.79	81.21–83.93	79.73–82.40	1.425–1.444
	Mean ± SD	68.0 ± 22.52	0.072 ± 0.02	1.493 ± 0.00	17.31 ± 1.38	82.69 ± 1.38	81.18 ± 1.35	1.435 ± 0.01
	CV	33.13	30.52	0.24	7.95	1.66	1.66	0.64

WIS—water-insoluble matter. RI—refractive index. M—moisture. SS—solid substances. TSS—total soluble substances. SG—specific gravity. SD-standard deviation; CV-coefficient of variation.

The results (color, water insoluble solids, refractive index, moisture, solid substances content, total soluble solids, specific gravity) are presented in Table 2.

Table 2. Parameters (pH, free acidity, ash, electrical conductivity, total phenols content, total flavonoids content) of honey samples.

Type	No Samples	Descriptive Statistics	pH	FA meq kg^{-1}	Ash %	EC mS cm^{-1}	TPC mgGAE/100 g	TFC mgQE/100 g
Acacia	**8**	Min–Max	4.14–4.72	6.8–15.4	0.040–0.100	0.130–0.220	11.10–17.92	0.44–1.63
		Mean ± SD	4.36 ± 0.18	11.3 ± 2.82	0.066 ± 0.02	0.173 ± 0.03	13.88 ± 2.39	0.86 ± 0.40
		CV	4.21	24.84	32.02	17.83	17.21	46.92
Linden	7	Min–Max	4.14–4.81	12.5–37.2	0.157–0.333	0.397–0.623	20.30–29.29	1.01–3.14
		Mean ± SD	4.42 ± 0.24	27.7 ± 7.93	0.246 ± 0.06	0.506 ± 0.09	24.37 ± 3.08	2.02 ± 0.78
		CV	5.51	28.65	25.99	17.03	12.65	38.70
Rapeseed	5	Min–Max	3.62–4.26	19.9–44.0	0.085–0.135	0.197–0.290	19.70–24.74	1.33–3.12
		Mean ± SD	4.00 ± 0.25	29.1 ± 9.62	0.101 ± 0.02	0.224 ± 0.04	21.72 ± 1.98	2.00 ± 0.69
		CV	6.17	33.05	19.84	16.88	9.10	34.42
Sunflower	5	Min–Max	3.25–5.03	21.6–47.0	0.127–0.428	0.328–0.637	20.60–28.84	1.63–3.92
		Mean ± SD	4.09 ± 0.67	28.8 ± 10.43	0.251 ± 0.11	0.428 ± 0.12	25.12 ± 3.26	2.52 ± 0.90
		CV	16.45	36.18	44.50	28.04	12.96	35.64
Mint	3	Min–Max	3.80–4.20	24.3–40.0	0.134–0.238	0.220–0.551	42.06–50.82	2.04–3.97
		Mean ± SD	4.02 ± 0.21	30.6 ± 8.29	0.202 ± 0.06	0.394 ± 0.17	47.20 ± 4.58	3.05 ± 0.97
		CV	5.16	27.09	29.17	42.17	9.70	31.71

WIS—water-insoluble matter. RI—refractive index. M—moisture. SS—solid substances. TSS—total soluble substances. SG—specific gravity. SD-standard deviation; CV-coefficient of variation.

3.2. Correlations between Physicochemicals Parameters

Correlations between analyzed parameters are presented in Figures 1 and 2

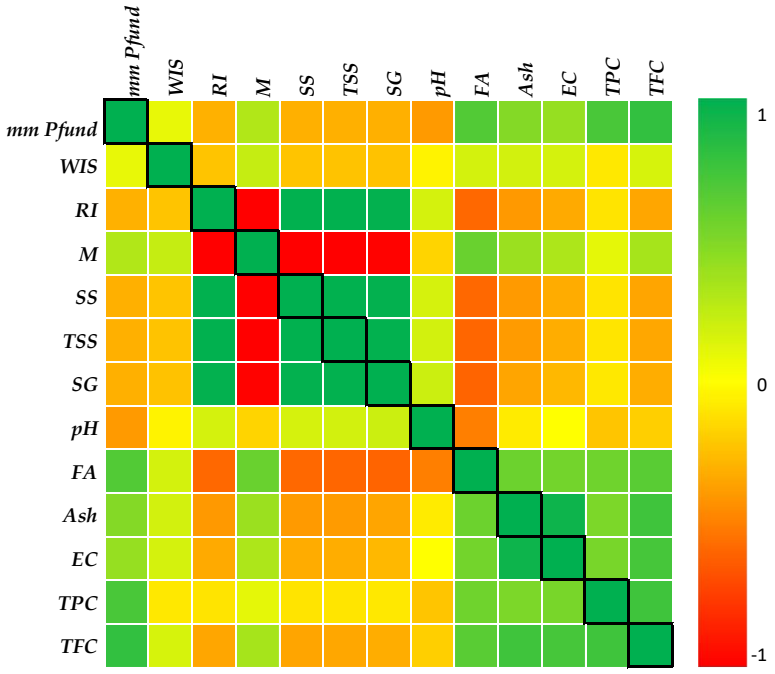
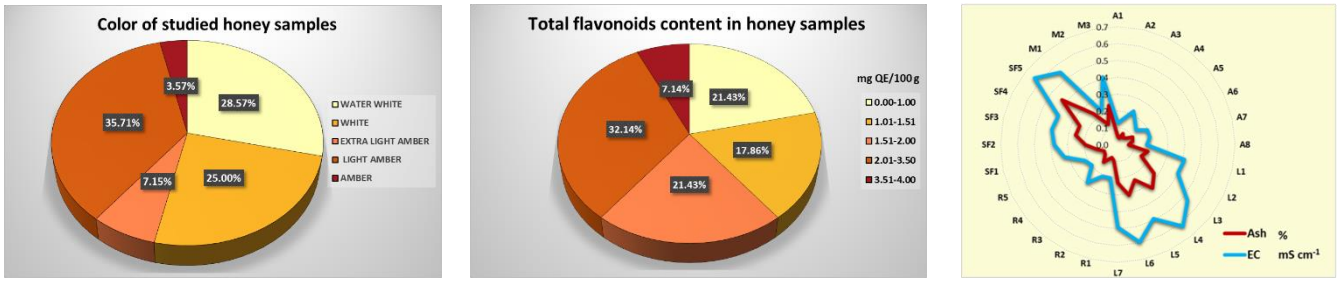


Figure 1. Head map of Pearson's correlation between honey parameters.



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

Figure 2. (**a**) Correlation between color and total flavonoids content; (**b**) Correlation between Ash content and electrical conductivity.

4. Discussion

Color of honey samples variated from water white (0.2 mm Pfund) in acacia honey sample to amber (86.1 mm Pfund) in mint honey sample. Water insoluble solids (WIS) varied from 0.035% in acacia honey to 0.114% in sunflower honey. Seven samples of raw monofloral honey (2 acacia, 2 linden, 2 rapeseed, 1 sunflower) had WIS content between 0.101% and 0.114%, over the level content of established by the legislation [19] of 0.1%, The refractive index (RI) read on the refractometer had values from 1.486 in rapeseed honey sample to 1.498 in acacia honey sample. The range of moisture content (M) and solid substances content (SS) were found between 15.41%, respectively 84.59% at acacia honey sample and 20.07%, respectively 79.93% at rapeseed honey sample (Table 1). For total soluble solids and specific gravity, minimum/maximum values of 78.28 °Brix/83.06 °Brix respectively, of 1.410 g/cm^3/1.448 g/cm^3 were found at rapeseed honey sample/acacia honey sample. The values of pH were found into 3.25–5.03 interval and for free acidity the results were under 50 meq kg^{-1}, value established by the legislation [19]. The highest average ash content has sunflower honey samples of 0.251% and of 0.066% the lowest in acacia samples. The values of electrical conductivity ranged between 0.130 mS cm^{-1} in acacia samples and 0.637 mS cm^{-1} and these findings revealed that the samples are floral originated honeys (less than 0.8 mS cm^{-1} established by Directive 2001/110/EC of the European Union for floral honey) [19]. The amount of total phenols content (11.10 mgGAE/100 g–50.82 mgGAE/100 g) and total flavonoids content (0.44 mgQE/100 g–3.97 mgQE/100 g) (Table 2) confirmed the antioxidant character of honey. The Pearson correlation coefficients between parameters of the analyzed honey samples are shown in Figure 1. There are some strong positive linear correlations between parameters: refractive index with solid substances (r = +1); total soluble solids (r = +1) and specific gravity (r = +0.99); color with: total phenols content (r = +0.72); total flavonoids content (r = +0.81) (Figure 2a). Strong correlations of total flavonoids content with ash (r = +0.76), electrical conductivity (r = +0.73) and total phenols content (r = +0.76), ash with electrical conductivity (r = +0.81) (Figure 2. b); between solid substances with total soluble solids (r = +1) and specific gravity (r = +0.99). Moderate positive linear correlation between color with free acidity (r = 0.68) and ash (r = 0.49). Moderate positive linear correlation was calculated between free acidity with 4 parameters: ash (r = 0.58); electrical conductivity (r = 0.55); total phenols content (r = 0.57) and total flavonoids content (r = 0.65); total phenols content is moderate positive correlated with ash (r = 0.51)and electrical conductivity (r = 0.53); Other research found similar correlations: Pontis et al. (2014) showed strong correlations between total phenolic content and color (r = 0.967), total flavonoids content and color (r = 0.924) [8] and between the same honey parameters, Al Farsi et al. (2018) found strong correlation with Pearson coefficient values up to 0.95 [9]. Total flavonoids content showed significant correlation with color (r = 0.82) in Almeida et al. research [20]. Cimpoiu et al. (2013) [11] obtained between total phenolic content and the color intensity in Romanian honey high linear correlation value of 0.8569. The investigated raw honey samples confirm the quality of honey produced in Romania.

5. Conclusions

Romanian honey is appreciated for its quality, this fact is proved by the large amount of honey exported.

The relevant levels of polyphenols and flavonoids identified in the analyzed honey demonstrate its antioxidant potential, as essential nutritional and sanogenic features in human nutrition.

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