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CORRELATION BETWEEN TOTAL PHENOL AND FLAVONOID CONTENT WITH SOME PHISICO-CHEMICAL PARAMETERS OF MONOFLORAL ROMANIAN HONEY

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Abstract

Twenty-four samples of monofloral Romanian honey (acacia, linden, rapeseed and sunflower) were analysed for their total phenolic and flavonoid contents, pH, free acidity, ash, electrical conductivity and color intensity. The analyses were performed in accordance with Romanian and EU standards and according to the methods in the literature. The results for color varied between 0.9-69.1 mm Pfund, for pH and free acidity between 3.54-4.44 and 4.7-15.7 meq kg^{-1}, respectively. The values of ash and electrical conductivity were between 0.043-0.291% and 0.12-0.55 mS cm^{-1}. The total phenolic content ranged from 14.50 mg GAE/100 g to 30.13 mg GAE/100 g while total flavonoid content ranged from 0.59 mg Q/100 g to 2.84 mg Q/100 g. The Pearson correlation analysis indicates positive significant correlations between color and total flavonoid content, ash and electrical conductivity, ash and total polyphenols content.

Key words*: correlation, honey, total flavonoid, total phenol.*

INTRODUCTION

The sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of plants or excretions of plant-sucking insects on the living parts of plants it is called honey (European Commission, 2002).
This viscous liquid is considered a complete food because contains many substances: carbohydrates, water, protein, minerals, vitamins and antioxidants (Halouzka, 2016).
The organic acids of honey give it an acidic character specific important for its preservation.
The diversity of minerals from honey contribute to the nutritional value. Many studies showed that in darker honeys there is a greater amount of minerals (De-Melo et al., 2017; Karabagias et al., 2017).
The amount of the component substances from honey depends on the environment from which it is harvested, therefore, honey is considered an environmental bioindicator (da Silva et al., 2016; De-Melo et al., 2017).
Phenolic compounds (flavonoids, phenolic acids), are responsible for the antioxidant activity of honey with favorable effects for human health (Alvarez-Suarez et al., 2010; Bogdanov, 2015).
For the antioxidant, antimicrobial and other beneficial properties in human health, honey is consumed as food or used as an ingredient (da Silva et al., 2016; De-Melo et al., 2017).
The aim of this study was to evaluate some quality parameters of honey samples and to assess the correlation between the investigated parameters.

MATERIALS AND METHODS

Twenty-four samples of monofloral honey were collected in 2017 from beekeepers at different sites in Iasi county. The six samples of each acacia (AH), linden (LH), rapeseed (RH) and sunflower (SFH) were analysed for their total phenolic and flavonoid content, pH, free acidity, ash, electrical conductivity and color intensity.
Samples were stored in laboratory at 20 ± 3°C in the dark. All analyses were done in accordance with Romanian, EU standards or according to the methods in the literature.
Shimadzu UV-mini-1240 spectrophotometer was used to determine the color of honey

samples. A 50% honey aqueous solution (w/v) was measured at 635 nm. The color of honey samples, was established after conversion of the absorbance values in mm Pfund (Table 1) (Ferreira et al., 2009; Pontis et al., 2014; Sant'ana et al., 2014).

Table 1. Pfund scale for determining color*

Color	Pfund scale (mm)
Water white	1 to 8
Extra white	8-17
White	17-34
Extra light amber	34-50
Light amber	50-85
Amber	85-114
Dark amber	More than 114

*Sereia et al. (2017)

A 10% (w/v) honey solution was prepared to determine the pH with WTW MULTI 3320 multiparameter (Bogdanov, 2009).

Free acidity was determined on a 10% (w/v) honey solution titrated with 0.1 N NaOH using TITRONIC universal-SCHOTT Instruments (Popescu & Meica, 1997; Standard Roman, 2009). The results were expressed in meq kg^{-1}.

The mineral content of honey samples was determined by calcination of samples in a muffle furnace (SUPERTHERM) at 550°C (Cantarelli et al., 2008; Popescu & Meica, 1997). The results were expressed in percentages (g/100 g).

The electrical conductivity was measured on a 20% (w/w) honey solution (dry matter basis) with WTW MULTI 3320 multiparameter (Bogdanov, 2009; Popescu & Meica, 1997). The results were expressed in mS cm^{-1}.

The total phenolic content (TPC) was determined by using Folin-Ciocalteu method, modified from Bobiș et al. (2008). The absorbance was measured at 742 nm against a blank with UV-1400 SHIMADZU Spectrophotometer. The calibration curve was made in 5 calibration points (y=0.0967x+0.083; R^2=0.9972) with gallic acid. The results were expressed in mg of gallic acid equivalents (GAE)/100 g.

Total flavonoid content was determined using aluminum chloride. The absorbance was measured at 430 nm (UV-1400 SHIMADZU Spectrophotometer). A standard calibration curve of quercetin was obtained in 5 calibration points (y=0.1326x-0.0123; R^2=0.9990). The results were expressed in mg of quercetin (Q)/100 g (Özkök et al., 2010; Pontis et al., 2014).

Analyses were made in triplicate. The data obtained were processed statistically with SPSS Statistical version 26.0. Correlations were tested by using Pearson's correlation coefficient (r) between the investigated parameters.

RESULTS AND DISCUSSIONS

Honey impressed consumers with its color, flavour and taste. The variety of colors is related to floral and geographical origin, content of polyphenols and flavonoids, storage time (Cimpoiu et al., 2013; De-Melo et al., 2017; Sereia et al., 2017).

The color intensity of all investigated honey samples is shown in Figure 2.

The results showed that the acacia samples have the lightest color-water white, with a maximum of 3.5 mm Pfund at AH1 sample (Table 2).

The most varied colors were observed for the sunflower honey samples, from white to light amber (Figure 1).

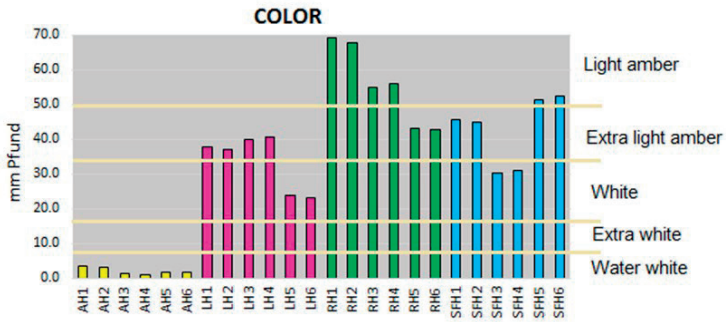

Figure 1. Color of honey samples

Table 2. Descriptive statistics of mm Pfund, pH and Free Acidity of honey samples

Type	n	Descriptive statistics	mm Pfund	Color	pH	Free Acidity (meq kg^{-1})
AH	6	Min.-Max.	0.9-3.5	water white	3.54-3.75	4.7-7.1
		Mean±SD	2.0±1.08		3.65±0.09	6.1±0.92
		CV	54.59		2.57	15.05
LH	6	Min.-Max.	23.1-40.8	white-extra light amber	3.83-4.44	11.4-13.5
		Mean±SD	33.7±8.14		4.14±0.11	12.4±0.84
		CV	24.13		6.56	6.77
RH	6	Min.-Max.	42.8-69.1	extra light amber-light amber	3.59-3.96	9.2-11.2
		Mean±SD	55.7±20.51		3.82±0.18	10.1±0.83
		CV	9.15		4.69	8.22
SFH	6	Min.-Max.	30.3-52.3	white-light amber	4.00-4.07	14.4-15.7
		Mean±SD	44.8±12.06		4.04±0.04	15.00±0.42
		CV	26.92		0.95	2.78

n - no. samples; SD - standard deviation; CV - coefficient of variation

The color in honey samples varied from 0.9 mm Pfund in acacia sample (AH1) to 69.1 mm Pfund in rapeseed sample (RH1) (Figure 1). The highest mean value of intensity of color was found for the rapeseed honey type, of 55.7 mm Pfund (Table 2).

Honey is an acidic food. pH values are found in the range 3.54-4.44. Acacia honey samples recorded the lowest pH value (Table 2). The high values of free acidity indicate a fermentation process. Maximum allowed value accepted and request in legislation is 50 milliequivalents acid per 1000 g (European Commission, 2002). The highest mean value of free acidity of 15.0 meq kg^{-1} was found for the sunflower honey (Table. 2).

Low values of pH and free acidity inhibit the growth of microorganisms, ensure product stability and a longer storage time (Pascual-Maté et al., 2018; Popescu & Meica, 1997).

The lowest mean value of ash of 0.060% was found for acacia honey samples and the highest mean value of ash was found for linden honey samples, 0.240% (Table 3).

Table 3. Descriptive statistics of ash, electrical conductivity, total polyphenols content and total flavonoids content of honey samples

Type	n	Descriptive statistics	Ash (%)	EC (mS cm^{-1})	TPC (mg GAE/100g)	TFC (mg Q/100g)
AH	6	Min.-Max.	0.043-0.076	0.12-0.16	14.50-16.80	0.59-0.79
		Mean±SD	0.060±0.01	0.15±0.02	15.65±0.93	0.66±0.07
		CV	20.94	10.64	5.96	10.68
LH	6	Min.-Max.	0.176-0.291	0.43-0.55	26.12-30.13	1.93-2.59
		Mean±SD	0.240±0.04	0.50±0.05	28.41±1.77	2.32±0.28
		CV	18.71	10.54	6.24	12.09
RH	6	Min.-Max.	0.046-0.100	0.18-0.21	17.02-25.51	1.85-2.52
		Mean±SD	0.074±0.02	0.19±0.02	21.26±3.72	2.20±0.27
		CV	26.05	8.43	17.51	12.39
SFH	6	Min.-Max.	0.155-0.278	0.40-0.47	19.24-28.05	1.83-2.84
		Mean±SD	0.213±0.05	0.42±0.043	24.02±3.95	2.39±0.45
		CV	24.62	6.08	16.46	18.66

n - no. samples; EC - electrical conductivity; TPC - total polyphenols content: TFC - total flavonoids content; SD - standard deviation; CV - coefficient of variation

Acacia samples registered the minimum mean value of electrical conductivity of 0.15 mS cm^{-1} and the maximum mean value of 0.50 mS cm^{-1}. All the values do not exceed the recommended limit value (0.8 mS cm^{-1}) (European Commission, 2002) and indicate the floral origin of investigated honey samples.

The climatic conditions, the botanical origin of honey, the season determined the content of polyphenolic compounds that give antioxidant properties of honey (Soares et al., 2017).

Polyphenolic compounds are mainly responsible for the antioxidant properties of honey. The content of these compounds depends on season, climatic conditions and mostly on the botanical origin of honey (Soares et al., 2017). The lowest value of total phenolic content was 14.50 mg GAE/100 g for one acacia honey sample and the highest value of 30.13 mg GAE/100 g was found for a linden honey sample (Table 3). The total flavonoid content ranged from 0.59 mg Q/100 g to 2.84 mg Q/100 g, with the highest value for sunflower honey (Table 3).

Similar studies on honey samples on the same parameters, from different countries, showed various values (Tables 4 and 5).

Table 4. Some parameters of acacia, linden, and polyfloral honey in literature

Country	pH	Free acidity (meq kg^{-1})	Ash	EC (mS cm^{-1})	References
Acacia					
Serbia	3.49-5.85	7.8-29.6	-	0.1-0.68	Lazarević et al., 2012
Romania	3.65-4.63	1.84-10.87	-	0.097-0.35	Stihi et al., 2016, Mărghitaș et al., 2010; Popescu et al., 2015, Scripcă et al., 2019
Poland	3.79	25.6	-	0.42	Tomczyk et al., 2019
Slovakia	3.71	16.1	-	0.20	Tomczyk et al., 2019
Linden					
Poland	3.81-4.13	14.5-34.2	-	0.53-0.579	Kędzierska-Matysek et al., 2018; Tomczyk et al., 2019
Serbia	3.98-5.40	8.2-26.2	-	0,3-0,76	Lazarević et al., 2012
România	3.6-4.7	-	0.186	0.20-0.73	Stihi et al., 2016; Popescu et al., 2015; Purcarea et al., 2016
Slovakia	3.90	21.6	-	0.23	Tomczyk et al., 2019
Rapeseed					
Slovakia	-	19	0.516	-	Kasperová et al., 2012
Romania	3,91-3,93	-	0.159-0.163	-	Stihi et al., 2016
Romania	4,22	16	0.162	-	Pauliuc et al., 2020
Sunflower					
Portugal	3,84	25.5	0,235	0.15	Aazza și colab., 2013
Poland	3,96	18.5	0.361	-	Kędzierska-Matysek et al., 2018
Serbia	3,17-4,14	11-42.7	0.19-0.55	-	Lazarević et al., 2012

EC=electrical conductivity

Table 5. Total polyphenols and flavonoids content of acacia, linden, rapeseed and sunflower honey in literature

Country	TPC (mg GAE kg^{-1})	TFC (mg QE kg^{-1})	References
Linden			
Czech Republic	450.37-730.09	18.79-35.61	Halouzka et al., 2016
Romania	160-380	47-69.8	Mărghitaș et al., 2009
Slovakia	350	2.57	Tomczyk et al., 2019
Acacia			
Czech Republica	238.36	8.73	Halouzka et al., 2016
Romania	120-260	8.4-32	Mărghitaș et al., 2010
Slovakia	200	1.37	Tomczyk et al., 2019
Rapeseed			
Czech Republic	94.3-119.2	5.8-6.1	Lachman et al., 2010
Romania	199	202	Pauliuc et al., 2020
Slovakia	210	2.16	Tomczyk et al., 2019
Sunflower			
Portugal	366.9	19.3	Aazza et al., 2013
Turkey	776.4	-	Gül & Pehlivan, 2018
Romania	200.0-450.0	115.3-153.3	Mărghitaș et al., 2009
Turkey	309.2-690.8	-	Özkök & Silici, 2018
Romania	8-16	-	Cimpoiu et al., 2013
Romania	211	228	Pauliuc et al., 2020

TPC= total polyphenols content, TFC= total flavonoids content

Principal Component Analysis of honey samples is presented in Figure 2. The samples of honey bee grouping on the type according to the studied parameters is highlighted. The best visual is the acacia honey samples group (Figure 3) by forming a well-highlighted cluster.

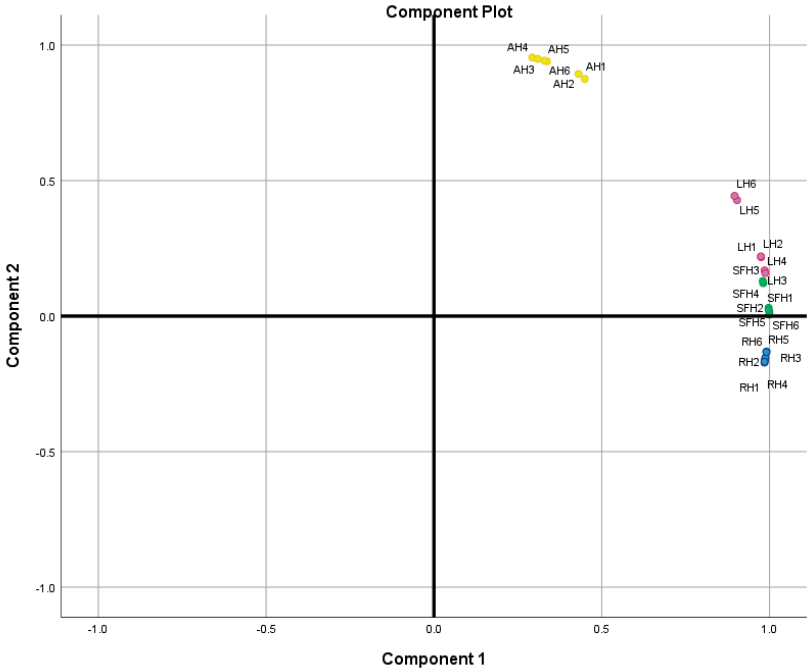

Figure 2. Principal Component Analysis of honey samples

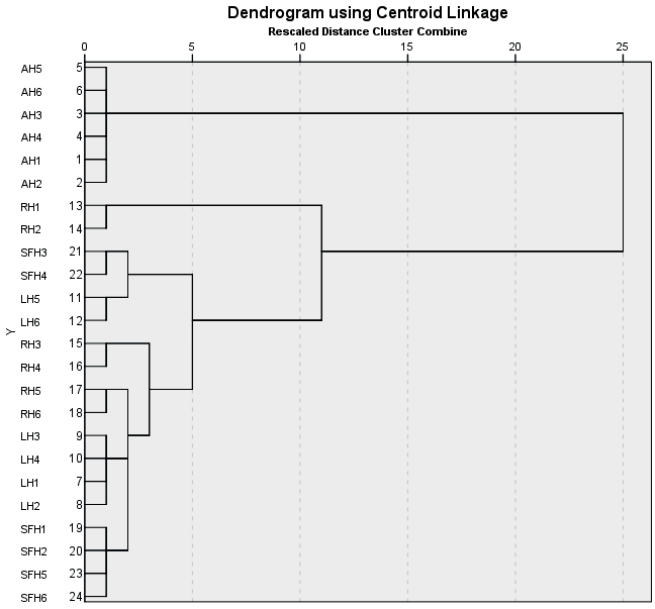

Figure 3. Hierarchical cluster analysis based studied honey parameters

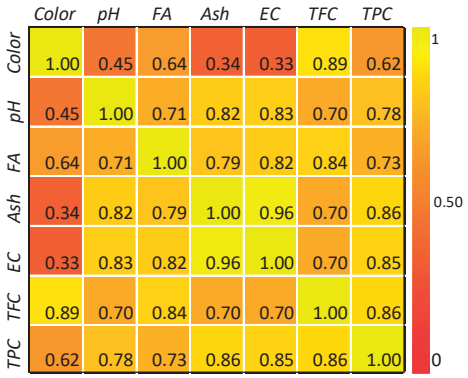

Figure 4. Pearson correlation coefficients of honey samples parameters

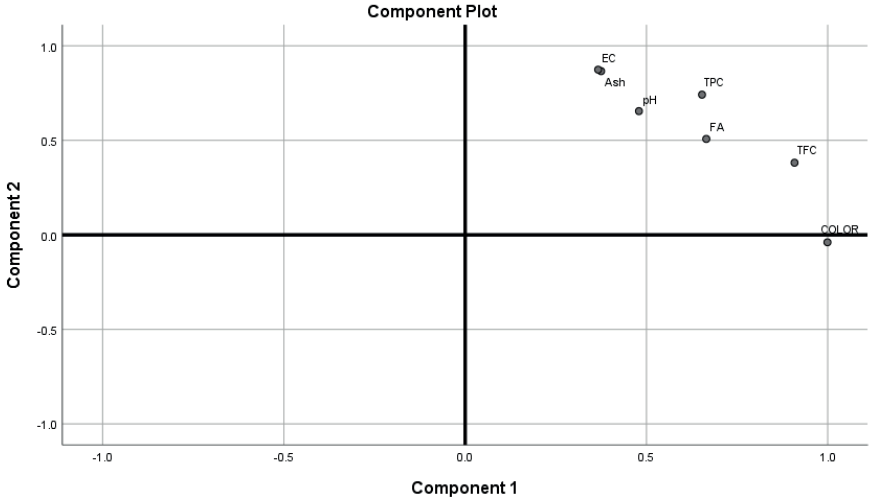

Figure 5. Principal Component Analysis of TPC, TFC and some physicochemical parameters of honey samples

Pearson correlation coefficients between the investigated parameters of twenty four honey samples is shown in Figure 4. The results of Pearson coefficient show strong positive linear correlations between ash content and electrical conductivity (r = 0.96) (Figure 5). A strong positive linear correlation (r = 0.89) was found between mm Pfund and total flavonoids content, between ash and total phenolic content, between total phenolic content and total flavonoids content, respectively (r = 0.86). Correlation Pearson value of 0.62 indicate a medium positive correlation between color and total phenolic content, of 0.64 between color and free acidity. Low positive correlation was found between pH and color (r = 0.45) and color and electrical conductivity (r = 0.33) (Figure 4) as represented in Figure 5.

Several investigations on different honey samples showed correlations between physicochemical parameters. Pontis et al. (2014) showed strong correlations between total phenolic content and color (r = 0.967), total flavonoids content and color (r = 0.924) and between total phenolic content and total flavonoids content (r = 0.926). Al Farsi et al. (2018) found strong correlation between same honey parameters, with Pearson coefficient values of 0.974, 0.999 and 0.977, respectively. Pearson coefficient of 0.75 between ash and electrical conductivity was reported by Ahmida et al. (2013); total flavonoids content showed significant correlation with color (r = 0.82) as shown by Almeida et al. (2016). High linear correlation value of 0.8569 and of 0.963 was obtained by Cimpoiu et al. (2013) between total

phenolic content and the color intensity in Romanian honey and by Kek et al. (2014) in malaysian honey, respectively.

CONCLUSIONS

Strong positive correlations are noticed between ash and electrical conductivity, color and total flavonoid content, between total phenolic content and total flavonoid content.
Medium positive correlations were found between color and free acidity, color and total flavonoid content.
Some parameters are not significantly correlated, with Pearson coefficients below 0,30: color with pH, ash, and with electrical conductivity, respectively.
The quality of honey bee depends on the geographical area, the floral origin, the climatic conditions but also on the health of the bee family.

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