



# **ASSESSMENT OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS AND THEIR CORRELATION WITH SOME PHYSICOCHEMICAL PARAMETERS OF MONOFLORAL ROMANIAN HONEY**

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# ABSTRACT

Since ancient times, honey has been considered not only a sweet food but also known as a remedy against diseases, due to its antioxidant properties.

The purpose of this study was to assess the total phenolic and flavonoid contents from Romanian raw monofloral honey and to establish their correlations with several qualitative parameters. In 2019, 28 samples were harvested: 8 acacia; 7 linden; 5 rapeseed, 5 sunflower and 3 mint, then analyzed in accordance with standardized methods, to measure: color intensity, water insoluble solids content, refractive index, moisture content, solid substances content, total soluble substances content, specific gravity, pH, free acidity, ash content, electrical conductivity, total phenols content and total flavonoids content.

Pearson test shown several correlation levels of total phenols content with other compounds: strong positive with total flavonoids ( $r=0.76$ ) and color intensity ( $r=0.72$ ); moderate positive with free acidity ( $r=0.57$ ), ash content ( $r=0.51$ ) and electrical conductivity ( $r=0.53$ ); weak negative with pH ( $r=-0.23$ ).

For total flavonoids content, correlations were: strong positive with color intensity ( $r=0.81$ ), ash content ( $r=0.76$ ) and electrical conductivity ( $r=0.73$ ); fairly strong positive with free acidity ( $r=0.65$ ); low positive between total flavonoids content and moisture ( $r=0.35$ ).

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

# Introduction

Honey bee has been used as food and also as medicine since ancient times. Properties of this beehive product like antimicrobial, anti-inflammatory, antioxidative activities have been recognized as having 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 condition. Antioxidant capacity of this complete food has been correlated with the amount of some substances that are present in honey composition: enzymes, polyphenolic compounds (phenolic acids, phenolic acid derivatives, flavonoids), proteins, amino acids and other compounds.

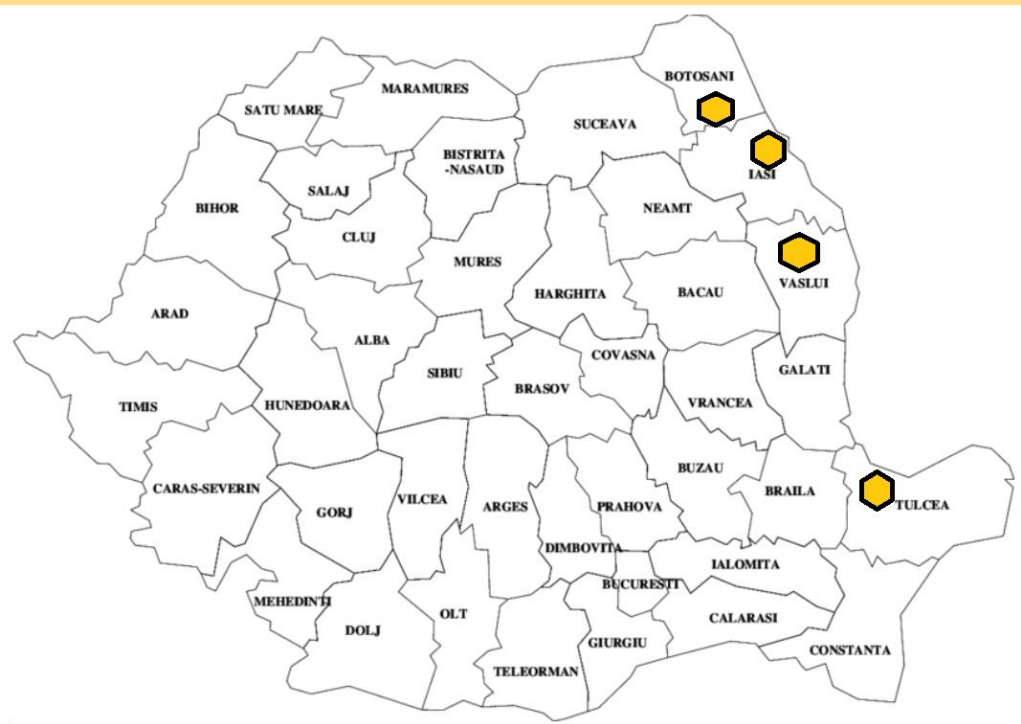
## Aim

The aim of this research was to assess total phenolic and total flavonoids contents from monofloral raw honey and to establish their correlations with several qualitative parameters.

# Materials and Methods



# ROMANIA



**RAPESEED  
5 SAMPLES  
(R)**

**ACACIA  
8 SAMPLES  
(A)**

**LINDEN  
7 SAMPLES  
(L)**

**SUNFLOWER  
5 SAMPLES  
(SF)**

**MINT  
3 SAMPLES  
(M)**

28 raw honey samples

4 counties: Botosani: A7, A8, L6, L7, R5, SF4, SF5

Iasi: A1, A2, A3, A4, L1, L2, R1, R2, SF1, SF2

Vaslui: A5, A6, L3, L4, L5, R3, R4, SF3

Tulcea: M1, M2, M3

<b>Parameter</b>	<b>Method</b>	<b>Reference</b>
<b>Color</b>	50% honey aqueous solutions were spectrophotometrically analyzed at 635 nm (Shimadzu UV-1700 Pharma Spec); the absorbance units were converted in mm Pfund	Ratiu I.A. et al., 2020
<b>Water insoluble solids (WIS)</b>	determined by the gravimetric method; the results were expressed in g/100 g honey	
<b>Refractive index (RI)</b>	read on the ABBÉ Kruss AR 2008 refractometer	SR(Romanian Standard) 784-3:2009: Honey bee. Part 3: Analytical methods
<b>Moisture (M)</b>	refractometric method (ABBÉ Kruss AR 2008 refractometer, with temperature correction); the results were expressed in g/100 g honey	
<b>Solid substances (SS)</b>	100- M (moisture); the results were expressed in g/100 g honey	
<b>Total soluble substances (TSS)</b>	refractometric method with ABBÉ Kruss AR 2008 refractometer; the results were expressed in °Brix	USDA, Extracted honey grading manual, United States Department of Agriculture. Standards for Honey Grading. USDA, Washington DC, 1985
<b>Specific gravity (SG)</b>	determined by the gravimetric method with pycnometer; the results were expressed in g/cm <sup>3</sup>	Popescu N. and Meica S., 1997

Parameter	Method	Reference
pH	10% (w/v) honey solution measured at WTW MULTI 3320 multiparameter	Bogdanov S., 2009; Sereia M.J. et al., 2017; SR(Romanian Standard) 784-3:2009: Honey bee.
Free acidity (FA)	titration method (10% (w/v) honey solution with 0.1 N NaOH), expressed in meq kg <sup>-1</sup>	Part 3: Analytical methods
Ash	calcination method (550 °C); the results were expressed in g/100 g	Bogdanov S., 2009; Sereia M.J. et al., 2017; SR(Romanian Standard) 784-3:2009: Honey bee.
Electrical conductivity (EC)	20% (w/w) honey solution (dry matter basis) in mili-Q water, measured at WTW MULTI 3320 multiparameter; the results were expressed in mS cm <sup>-1</sup>	Part 3: Analytical methods
Total phenols content (TPC)	Folin-Ciocalteu method modified from Bobiș et al. (2008) and Sereia et al. (2017). The absorbance was measured at 742 nm against a blank (UV-1400 Shimadzu spectrophotometer). Standard calibration (gallic acid; 5 calibration points and 3 replicates; concentration ranging in 2-12 mg L <sup>-1</sup> interval; $y=0.089x+0.1147$ ; $R^2=0.9972$ ). The results were expressed in mg of quercetin equivalents (QE)/100g	Bobiș et al. (2008) and Sereia et al. (2017)
Total phenols content (TPC)	method with minor changes developed by Bobiș et al. (2008) and Pontis et al. (2014). The absorbance was measured at 430 nm against a blank (UV-1400 Shimadzu spectrophotometer). Standard calibration (quercetin; 6 calibration points and 3 replicates; concentration ranging in 0.5-5 mg L <sup>-1</sup> interval; $y=0.1331x+0.0112$ ; $R^2=0.9997$ ). The results were expressed in mg of quercetin equivalents (QE)/100g	Bobiș et al. (2008) and Pontis et al. (2014)

# Results and Discussions



**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, [light blue]-minim-value; [green]-maxim-value

**(Council Directive 2001/110/CE concerning honey)**

**Moisture**  
Limit:  
< 20% moisture

**WIS**  
Impurity content for commercial honeys:  
< 0.1% for filtered honey  
< 0.5% for pressed honey

In present research  
Samples: 2 samples acacia, 2 samples linden, 2 samples rapeseed, 1 sample sunflower  
WIS>0.1%  
Sample: 1 sample rapeseed M>20%

**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 <sup>-1</sup>	Ash %	EC mS cm <sup>-1</sup>	TPC mgGAE/100g	TFC mgQE/100g
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, , -minim value; -maxim value

(Council Directive 2001/110/CE concerning honey)

**Free acidity**

Limit:  
< 50 meq kg<sup>-1</sup>

**Electrical conductivity**

Limit:  
< 0.8 mS cm<sup>-1</sup>

# Correlations between some honey parameters

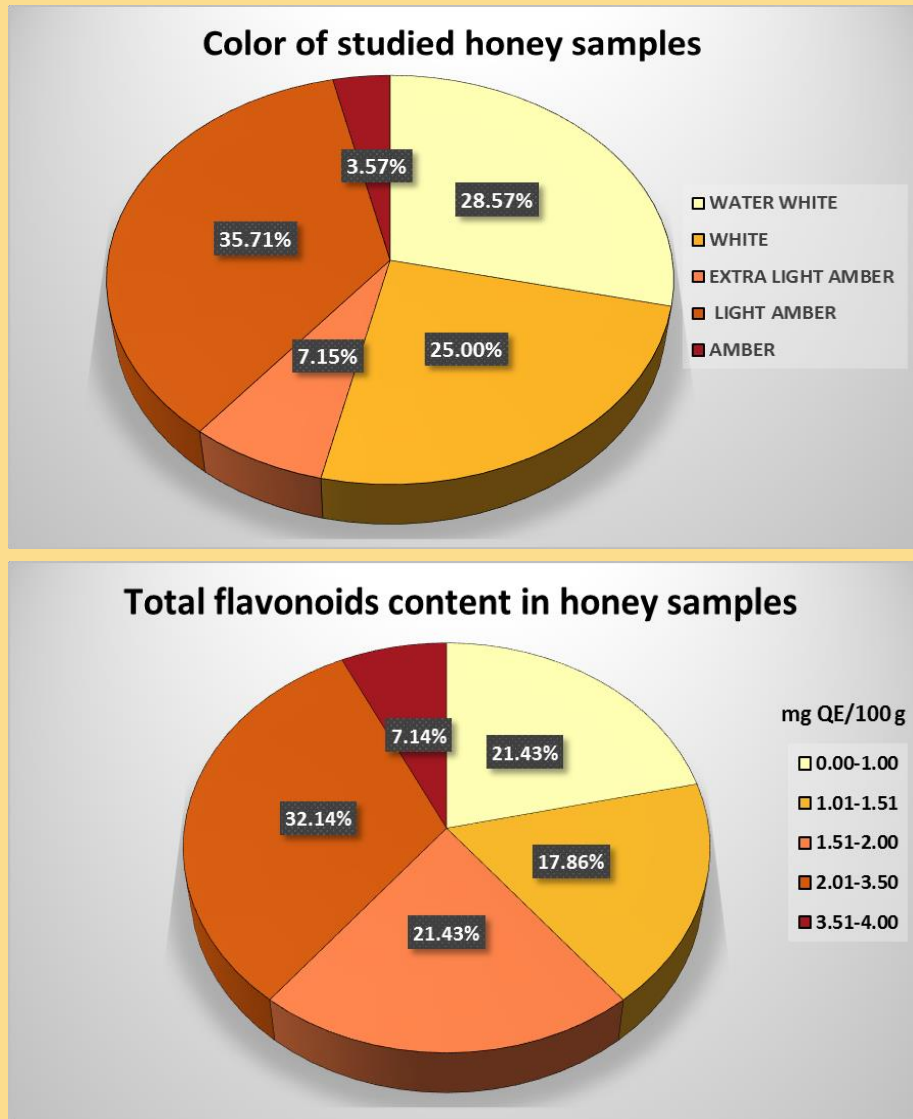


Figure 1. Correlation between color and total flavonoids content fi honey samples (r=0.81)

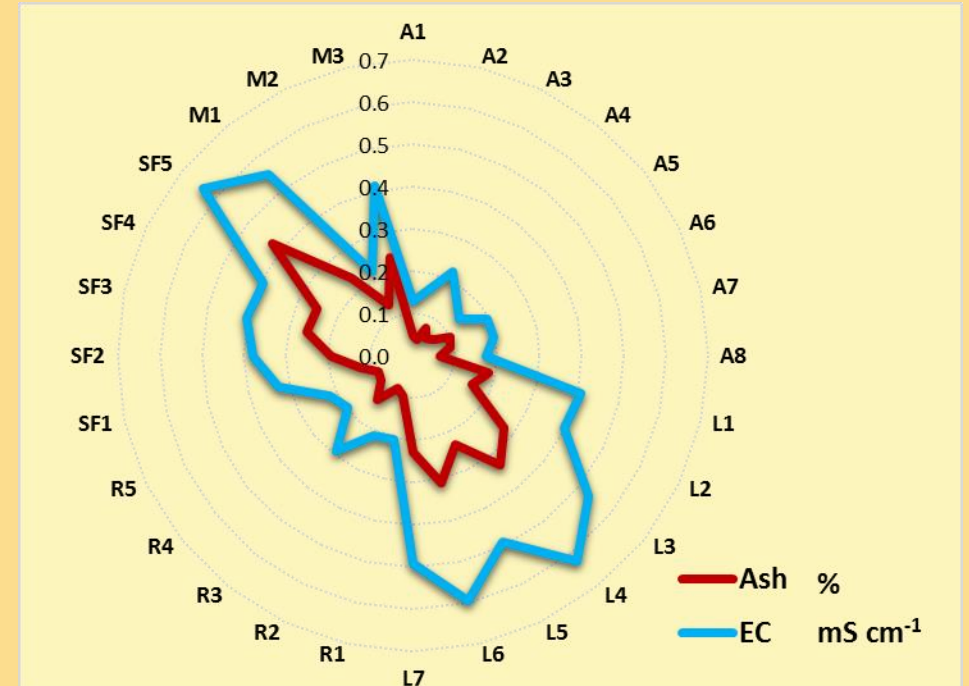
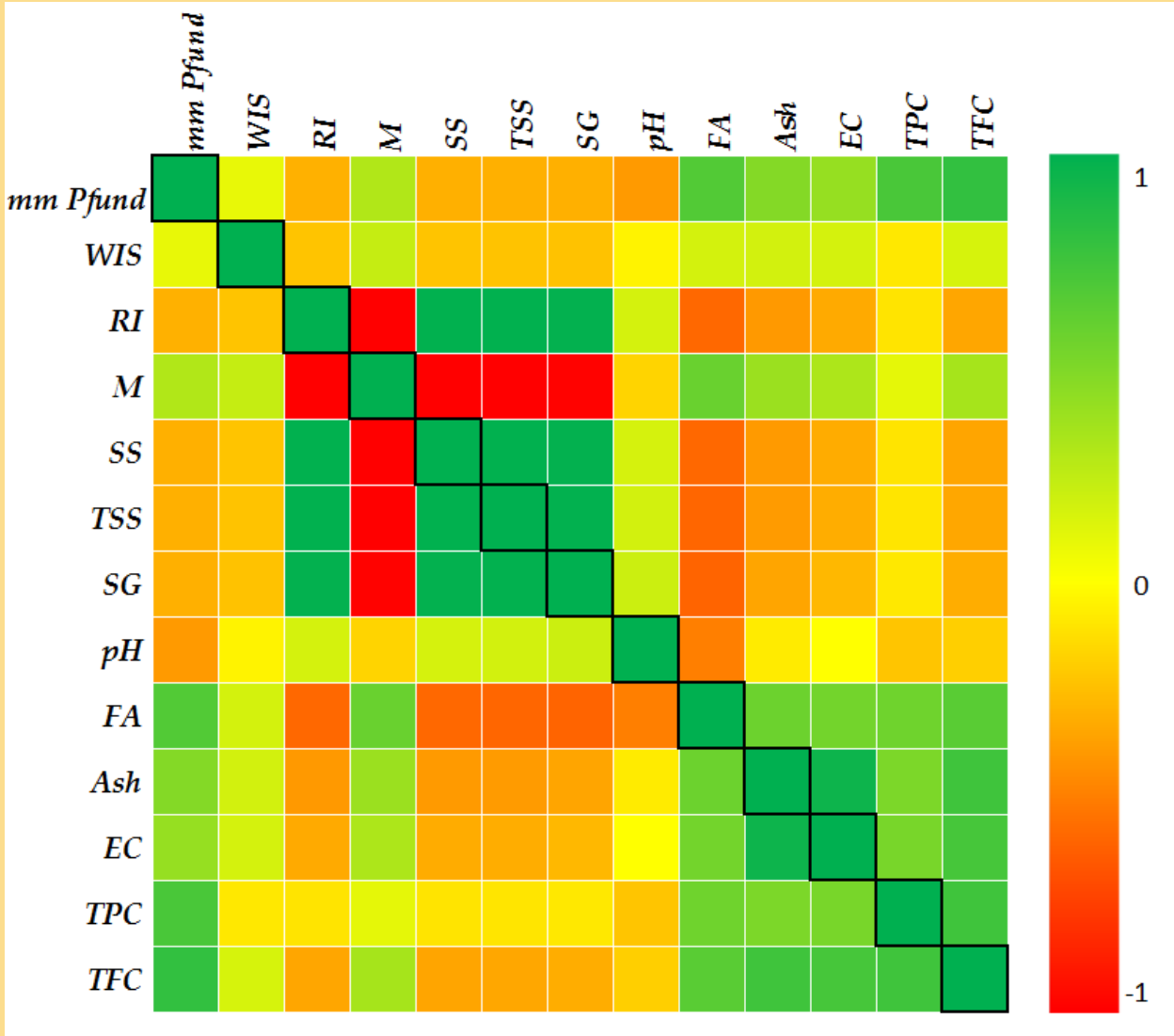


Figure 2. Correlation between ash and electrical conductivity (r=0.95)

# Pearson's correlation



## Correlations of honey parameters

Strong positive linear correlation between:

- RI and : SS ( $r=+1$ ); TSS ( $r=+1$ ); SG ( $r=+0.99$ );
- Color and: TPC ( $r=+0.72$ ); TFC ( $r=+0.81$ );
- TFC and: Ash ( $r=+0.76$ ); EC ( $r=+0.73$ ); TPC ( $r=+0.76$ );
- Ash and EC ( $r=+0.81$ );
- SS and: TSS ( $r=+1$ ); SG ( $r=+0.99$ );

Moderate positive linear correlation between:

- Color and : FA ( $r=0.68$ ); Ash ( $r=0.49$ );
- FA and: Ash ( $r=0.58$ ); EC ( $r=0.55$ ); TPC ( $r=0.57$ );  
TFC ( $r=0.65$ );
- TPC and: Ash ( $r=0.51$ ); EC ( $r=0.53$ );

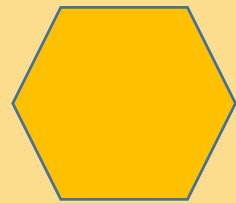
Figure 3. Heat map of Pearson's correlation between honey parameters

# 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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**THANK YOU**

