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PHYSICOCHEMICAL PARAMETERS AND SPECTRAL STRUCTURE (FT-IR) OF HONEY FROM IASI COUNTY (NORTH-EASTERN ROMANIA)

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Abstract

The aim of this study was to investigate some physicochemical parameters of honey from Iasi County (North-Eastern Romania). Twenty-four samples of four floral honey types (rapeseed, acacia, linden, polyfloral) were collected in 2016 from beekeepers. All analyses were performed according to both Romanian and EU standards. The results for colour, refractive index, total soluble solids, moisture and density varied between 1.12-69.70 mm Pfund, 1.4886-1.4948, 80.84-83.28 °Brix, 16.72-19.16% and 1.423-1.439 g cm^{-1}, respectively. The pH, free acidity, electrical conductivity and ash ranged between 3.84-4.75, 17.6-48.6 meq kg^{-1}, 166-794 µS cm^{-1} and 0.05-0.20%, respectively. The total phenolic content ranged from 16.10 mg GAE/100 g to 31.44 mg GAE/100 g while total flavonoid content ranged from 0.97 mg Q/100 g to 2.59 mg Q/100 g. For all honey samples the results revealed strong positive correlation between colour and total phenolic content with total flavonoid content, and between ash and electrical conductivity, respectively. The spectral structure (FT-IR) of studied honey samples showed the presence of various functional groups.

***Key words**: honey, physicochemical, FT-IR, NE Region, Romania.*

INTRODUCTION

According to EU legislation (European Commission, 2002) honey is defined as the natural sweet substance produced by *Apis mellifera* bees. Honey is a complex natural foodstuff and the only sweetening agent that can be used by humans without processing. The variety of the environmental forms, different weather conditions and a large variety of melliferous flora make Romania an important producer of honey in European Union (Popescu, 2017).

The composition and the properties of this product of beehive are considerably influenced by floral source, geographical and environmental factors (Halouzka et al., 2016; Mărghitaș et al., 2009). The highest amounts of components in honey consists of a complex mixture of sugars and water. Honey also contains other chemical compounds which, even at low concentrations, contribute to its high nutritional and curative properties (Arawwawala and Hewageegana, 2017; Ferreira et al., 2009). Polyphenolic compounds, such as flavonoids have been proved to give the antioxidant and antimicrobial activities of honey (Bridi et al., 2017; Ferreira et al., 2009; Kurtagić et al., 2013).

The main objective of this study was to investigate the quality of honey from some sites of Iasi county. Some parameters, such as moisture, total soluble solids, acidity, electrical conductivity can give information about the honey quality in terms of standard regulations (Isopescu et al., 2014).

The infrared spectroscopy technique was used to determine the spectral structure of the analysed samples.

MATERIALS AND METHODS

Twenty-four samples (three sub-samples from each type of honey) of four floral honey types (rapeseed - R1, R2, acacia - S1, S2, linden - T1, T2, polyfloral - P1, P2) were collected in 2016 from beekeepers at different sites of Iasi county (Figure 1).

Honey samples were stored in the dark in laboratory at 20-24°C. Three laboratory replicates were analysed for each sub-sample, according to Romanian standards (Standard Roman, 2009), harmonised methods of the International honey commission (Bogdanov,

2009) and European Union Honey Directive (European Commission, 2002).
The color was determined spectrophotometrically at 635 nm (Shimadzu UV-mini-1240) for a 50% honey aqueous solution (w/v). The honey samples were classified according to the Pfund scale (Table 1) after conversion of the absorbance values (Ferreira et al., 2009; Pontis et al., 2014; Rebiai & Lanez, 2014; Sant'ana et al., 2014).

Figure 1. The honey sampling sites

The refractive indices of honey samples were measured using an ABBÉ Kruss AR 2008 refractometer and corrected for the temperature of 20°C (Bogdanov, 2009; Rebiai & Lanez, 2014; Standard Roman, 2009). The moisture content, the total soluble solids and density were determined by the refractometer method using the values of refractive indices (Bogdanov, 2009; Crane, 1979; Popescu & Meica, 1997; Standard Roman, 2009; USDA, 1985).
pH was measured on a 10% (w/v) honey solution and the electrical conductivity on a 20% (w/w) honey solution (dry matter basis) with WTW MULTI 3320 multiparameter (Bogdanov, 2009; Popescu and Meica, 1997; Sereia et al., 2017).
Free acidity was determined by the titrimetric method. A 10% (w/v) honey solution was titrated with 0.1 N NaOH using TITRONIC universal-SCHOTT Instruments (Popescu and Meica, 1997; Standard Roman, 2009).
The ash content was determined by calcination of samples in a muffle furnace (SUPERTHERM) at 550°C (Cantarelli et al., 2008; Popescu and Meica, 1997).
The total phenolic content (TPC) was determined by using 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). Gallic acid was used as standard to obtain the calibration curve (5 calibration points; $y=0.0993x+0.0737$; $R^2=0.9991$). The results were expressed in mg of gallic acid equivalents (GAE)/100 g.
Total flavonoid content was determined by using a method with minor changes developed by Bobiș et al. (2008), Özkök et al. (2010) and Pontis et al. (2014).
The absorbance was measured at 430 nm against a blank (UV-1400 SHIMADZU Spectrophotometer). A standard calibration curve of quercetin was obtained in 5 calibration points ($y=0.1364x-0.0131$; $R^2=0.9997$). The results were expressed in mg of quercetin (Q)/100 g.
The structural identification of some compounds in honey samples was made by FTIR spectroscopy method with an Identify IR–Portable FT-IR spectrometer within a range from 4000 cm^{-1} to 650 cm^{-1}.
The statistical analyses were performed with the Statistica 12 and the FT-IR spectra were processed using Origin 8 software.

RESULTS AND DISCUSSION

Color of honey is not considered a quality parameter but has a direct visual impact on consumers and on the price.
The color variation is directly related to floral origin, mineral content, product and storage of honey, flavonoids content (Sereia et al., 2017).
The results showed that color of rapeseed honey samples is light amber and that of acacia honey is water white.
The color of linden honey samples (T1) is extra light amber and of T2 samples is white (Table 2).
The polyfloral samples also had different colors: P1 samples color was between extra light amber and light amber and P2 color samples was much lighter - white color.
The determination of moisture content is the most frequent analysis to evaluate the quality of honey. Any excess of water affects the physical properties of honey.

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

Water content is very important to avoid fermentation and increase storage time (Soares et al., 2017). National and international legislation recommend the water content in honey to be less than 20% (European Commission, 2002; Standard Roman, 2009). The moisture content in the analyzed honey samples ranged between 16.72% (polyfloral sample P1) and 19.16% (polyfloral sample P2) (Table 2). For all samples moisture content was below 20%. Stihi et al. (2016) reported four acacia honey samples from Romania with moisture content between 20% and 22.8%.

Total soluble solids are represented by sugars and depend on moisture content. When values are higher than 80°Brix (20% moisture) honey can be considered of high grade and stable during storage because as total soluble solids increase, moisture drops (Nyau et al., 2013, USDA, 1985). In honey samples values of total soluble solids ranged between 80.84-83.28°Brix (Table 2).

Density of honey is a parameter of practical significance, that could indicate the optimum amount of honey to be stored (honey with high water content is less dense). Polyfloral samples P2 were of the lowest density (1.423 g cm^{-1}) and the polyfloral honey samples P1 are the densest (1.439 g cm^{-1}).

Usually, the pH of honey varies between 3.5 and 5.5 (Popescu and Meica, 1997). The pH values of the analyzed honey samples were between 3.84 and 4.75 (Table 3). At low pH values the microbial activity is inhibited and the shelf life is extended (Krishnasree and Ukkuru, 2017).

Honey is an acid product. The free acidity is related to the freshness of honey. High acidity could indicate a possible fermentation. International legislation (European Commission, 2002) requires the highest limit value of 50 meq kg^{-1} free acidity.

The lowest values of free acidity were determined on acacia honey samples: S1 with 17.6 meq kg^{-1} and S2 with 18.9 meq kg^{-1} average value, respectively.

The polyfloral honey samples (P2) had the highest free acidity value (48.6 meq kg^{-1}).

Table 2. Physico-chemical parameters of honey samples

Type	n	Descriptive statistics	Color (mm Pfund)	RI	TSS (°Brix)	Moisture (%)	Density (g cm^{-1})
R1	3	Min-Max	67.52-72.35	1.4907-1.4914	81.68-81.96	18.04-18.32	1.429-1.431
		Mean±SD	69.70±1.69	1.4910±0.001	81.78±0.11	18.22±0.11	1.429±0.001
		CV	2.42	0.02	0.14	0.61	0.05
R2	3	Min-Max	55.63-61.58	1.4928-1.4943	82.52-83.08	16.92-17.48	1.434-1.438
		Mean±SD	58.15±2.26	1.4937±0.001	82.84±0.19	17.16±0.19	1.436±0.001
		CV	3.88	0.03	0.23	1.11	0.09
S1	3	Min-Max	3.27-4.01	1.4917-1.4929	82.08-82.56	17.44-17.92	1.432-1.434
		Mean±SD	3.51±0.32	1.4924±0.001	82.35±0.16	17.65±0.16	1.433±0.001
		CV	9.15	0.03	0.19	0.9	0.06
S2	3	Min-Max	1.04-1.41	1.4893-1.4903	81.08-81.52	18.48-18.92	1.425-1.428
		Mean±SD	1.12±0.16	1.4899±0.001	81.33±0.15	18.67±0.15	1.426±0.001
		CV	14.61	0.02	0.18	0.79	0.07
T1	3	Min-Max	37.06-40.41	1.4888-1.4897	80.92-81.28	18.72-19.08	1.424-1.426
		Mean±SD	38.80±1.02	1.4893±0.001	81.11±0.12	18.89±0.12	1.425±0.001
		CV	2.62	0.02	0.14	0.61	0.05
T2	3	Min-Max	29.64-31.86	1.4902-1.4915	81.46-82.00	18.00-18.54	1.427-1.438
		Mean±SD	31.04±0.85	1.4907±0.001	81.67±0.21	18.33±0.21	1.430±0.003
		CV	2.73	0.03	0.25	1.13	0.24
P1	3	Min-Max	48.21-51.92	1.4943-1.4952	83.11-83.44	16.56-16.89	1.438-1.440
		Mean±SD	49.81±1.27	1.4948±0.001	83.28±0.13	16.72±0.13	1.439±0.001
		CV	2.56	0.02	0.15	0.75	0.06
P2	3	Min-Max	29.64-31.86	1.4874-1.4903	80.36-81.54	18.46-19.64	1.420-1.428
		Mean±SD	30.87±0.83	1.4886±0.001	80.84±0.38	19.16±0.38	1.423±0.002
		CV	2.69	0.07	0.48	2.01	0.18

n-no. samples; RI-refractive index; TSS-Total Soluble Solids; SD-standard deviation; CV-coefficient of variation

For all honey samples the free acidity values are below the required limit of 50 meq kg^{-1} (Table 3).

Electrical conductivity depends directly on the ash content and has been replacing the ash content in the international standards. The electrical conductivity values in the analyzed honey samples varied in range of 166-794 µS cm^{-1}, and do not exceed the recommended limit value (0.8 mS cm^{-1}) (European Commission, 2002). The lowest values were observed for acacia honey samples (Table 3).

The mineral content of honey depends on the quality of the nectar collected by bees. The number and the content level of honey minerals and trace elements depend on botanical and geographical origins. The lowest ash content of 0.05% was found on acacia honey samples (S1). In honey samples from different regions of Romania, Mărghitaș et al. (2009) reported similar lowest values of 0.03-0.28% on acacia honey samples.

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 16.1 mg GAE/100 g for acacia honey samples (S1) and the highest value of 31.44 mg GAE/100 g was recorded for polyfloral samples (P1) (Table 4).

Table 3. Characteristic parameters (pH, free acidity, electrical conductivity, ash) of honey samples

Type	n	Descriptive statistics	pH	Free Acidity (meq kg^{-1})	EC (µS cm^{-1})	Ash (%)
R1	3	Min-Max	3.98-4.08	24.8-26.2	239-253	0.09-0.11
		Mean±SD	4.01±0.03	25.5±0.51	245±4.51	0.10±0.008
		CV	0.73	1.98	1.84	7.93
R2	3	Min-Max	4.08-4.12	22.3-23.5	222-234	0.07-0.08
		Mean±SD	4.10±0.01	23.0±0.38	230±4.04	0.07±0.005
		CV	0.33	1.65	1.76	6.32
S1	3	Min-Max	4.00-4.10	17.1-18.2	160-173	0.05-0.06
		Mean±SD	4.06±0.04	17.6±0.37	166±3.82	0.05±0.004
		CV	0.88	2.12	2.3	6.74
S2	3	Min-Max	3.75-3.91	18.5-19.3	249-262	0.10-0.13
		Mean±SD	3.84±0.06	18.9±0.26	254±3.97	0.11±0.009
		CV	1.65	1.39	1.56	8.4
T1	3	Min-Max	4.52-4.70	32.2-33.5	513-524	0.19-0.21
		Mean±SD	4.63±0.06	32.3±0.46	518±4.00	0.20±0.009
		CV	1.38	1.4	0.77	4.28
T2	3	Min-Max	4.63-4.86	43.4-45.1	500-516	0.15-0.20
		Mean±SD	4.75±0.07	44.1±0.58	509±4.18	0.18±0.018
		CV	1.51	1.31	0.82	9.98
P1	3	Min-Max	3.86-4.01	39.6-41.0	577-591	0.16-0.20
		Mean±SD	3.95±0.06	40.6±0.52	584±4.94	0.18±0.015
		CV	1.45	1.28	0.84	7.97
P2	3	Min-Max	4.32-4.47	47.3-49.7	785-800	0.19-0.21
		Mean±SD	4.40±0.05	48.6±0.86	794±4.89	0.20±0.009
		CV	1.13	1.77	0.62	4.52

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

Similar studies on honey samples from Romania showed various content of phenolic compounds: in linden honey samples were found from 16 mg GAE/100 g to 38 mg GAE/100 g, in acacia honey samples were found from 2 mg GAE/100 g to 39 mg GAE/100 g (Mărghitaș et al., 2009), in polyfloral honey samples were found 31 mg GAE/100 g and in linden honey samples were found 53 mg GAE/100 g (Dobre et al., 2010).

Flavonoids have antioxidant and anti-inflammatory properties. The total flavonoid content ranged from 0.97 mg Q/100 g to 2.59 mg Q/100 g, with the highest value for rapeseed honey (Table 4). The total flavonoids content of 4.7-6.9 mg Q/100 g for Romanian linden honey and of 0.9-2.4 mg Q/100 g on Romanian acacia honey were reported by Mărghitaș et al. (2009).

Several studies showed significant correlations between some characteristic parameters of honey. Pontis et al. (2014) found strong positive correlation between color intensity, flavonoid content and phenolic content; a positive correlation was observed by Ahmida et al. (2013), Sohaimy et al. (2015) between electrical conductivity and total ash content.

Table 4. Total phenols content and total flavonoids content of honey samples

Type	n	Descriptive statistics	TPC (mg GAE/100 g)	TFC (mg Q/100 g)	Type	n	Descriptive statistics	TPC (mg GAE/100 g)	TFC (mg Q/100 g)
R1	3	Min-Max	25.87-27.58	2.52-2.64	T1	3	Min-Max	29.43-30.63	2.00-2.17
		Mean±SD	26.80±0.56	2.59±0.05			Mean±SD	30.01±0.36	2.09±0.06
		CV	2.1	1.79			CV	1.19	2.77
R2	3	Min-Max	22.91-24.59	2.06-2.22	T2	3	Min-Max	22.24-23.59	1.48-1.55
		Mean±SD	23.91±0.60	2.12±0.05			Mean±SD	22.76±0.50	1.52±0.03
		CV	2.52	2.4			CV	2.18	1.67
S1	3	Min-Max	15.19-16.87	0.93-1.01	P1	3	Min-Max	30.90-31.98	2.24-2.34
		Mean±SD	16.10±0.55	0.97±0.03			Mean±SD	31.44±0.42	2.30±0.04
		CV	3.39	2.92			CV	1.32	1.53
S2	3	Min-Max	18.42-19.22	1.22-1.34	P2	3	Min-Max	29.34-30.85	1.85-1.99
		Mean±SD	18.90±0.28	1.28±0.04			Mean±SD	29.94±0.046	1.92±0.04
		CV	1.46	2.87			CV	1.54	2.2

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

Khalil et al. (2012) observed strong correlation between phenolic and flavonoid contents, color intensity and flavonoid content.

Krishnasree and Ukkuru (2017) noticed positive correlations between pH and moisture, pH and acidity, acidity and ash.

Table 5 presents the Pearson correlation coefficients between parameters of the analyzed honey samples.

A strong positive correlation was found between color intensity, total phenols content and total flavonoids content, between refractive indices, total soluble solids and density, between acidity, electrical conductivity and ash content, between electrical conductivity and total phenols content and between total phenols content and total flavonoids content.

A strong negative correlation is observed between moisture, refractive index, total soluble solids and density.

The factor analysis based on chemical composition of honey samples are presented in Figure 2.

The refractive index, the total soluble solids and the moisture content were the main variables determining the ranking of honeys on factor 1 (38.15% of variance accounted for), whereas the second factor (32.07% of variance accounted for) would be explained mainly by the free acidity, electrical conductivity and ash and the third factor (22.69% of variance) by color, total phenols content and total flavonoids content.

Table 5. Correlation matrix of honey samples parameters (Pearson correlation coefficients)

	Color	RI	TSS	Moisture	Density	pH	Acidity	EC	Ash	TPC	TFC
Color	1.00										
RI	0.31	1.00									
TSS	0.31	1.00	1.00								
Moisture	-0.31	-1.00	-1.00	1.00							
Density	0.30	0.99	0.99	-0.99	1.00						
pH	0.05	-0.48	-0.48	0.48	-0.40	1.00					
Acidity	0.24	-0.22	-0.22	0.22	-0.17	0.61	1.00				
EC	0.11	-0.34	-0.34	0.34	-0.31	0.53	0.94	1.00			
Ash	0.12	-0.41	-0.41	0.41	-0.37	0.62	0.87	0.92	1.00		
TPC	0.66	-0.05	-0.05	0.05	-0.06	0.27	0.68	0.74	0.74	1.00	
TFC	0.94	0.16	0.16	-0.16	0.14	-0.01	0.31	0.27	0.31	0.82	1.00

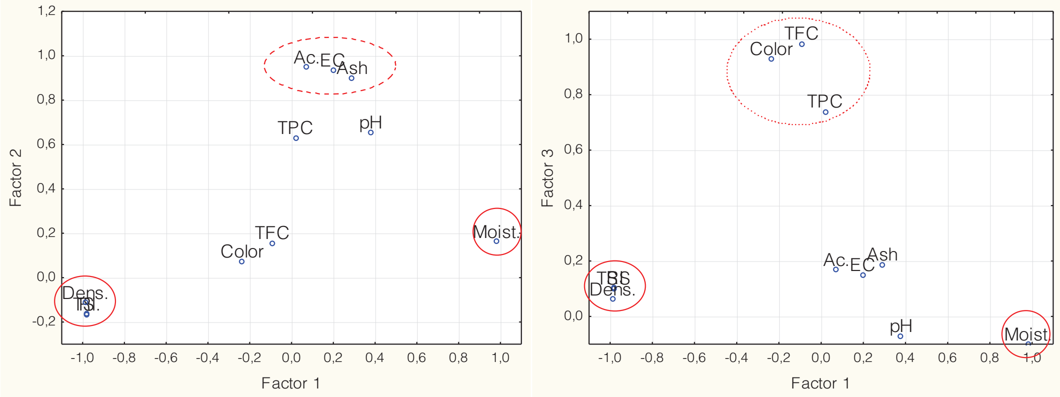

Figure 2. Discrimination of the most influential variables on the observed separations on the basis of factor analysis performed on data recorded on chemical composition of honey

FTIR spectroscopy is a rapid and non-destructive analytical method for quality control of honey through the spectrum obtained from 4000 cm^{-1} to 650 cm^{-1} spectral region. The spectrum shows peaks that correspond with the main classes of organic molecules (Anguebes et al., 2016; Nayik et al., 2015).

Moisture, carbohydrates and other minor compounds are mainly responsible for these variations in the spectral structural composition of honey samples (Figure 3).

There are some regions where the absorption bands were attributed to some bonds which belong to the structure of honey compounds.

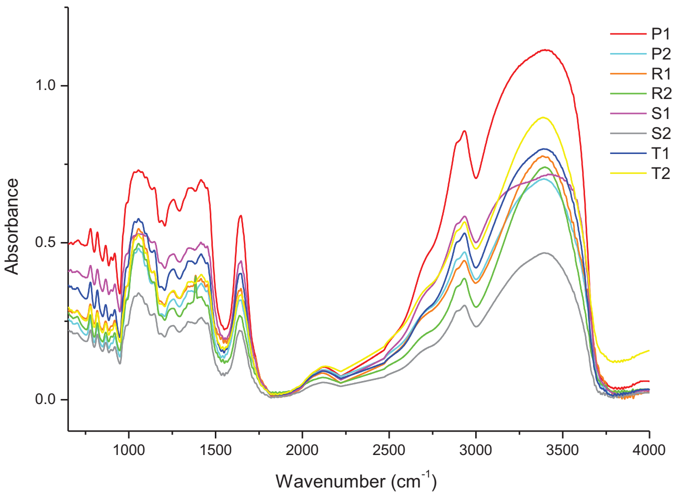

Figure 3. FTIR spectra of honey samples in the range 650-4000 cm^{-1}

The absorption bands between 3700 cm^{-1} and 3000 cm^{-1} are due to the stretching vibrations of the -OH functional group from carbohydrates, water and organic acids present in honey samples (Anguebes et al., 2016).

The band at 3000-2700 cm^{-1} corresponds to the stretching vibration of the C-H bonds which constitute the chemical structure of the carbohydrates, the stretching vibration of the O-H bonds of the carboxylic acids and NH_3 of free amino acids (Anguebes et al., 2016; Franca and Oliveira, 2011; Gok et al., 2015).

The band at 1700-1600 cm^{-1} was attributed to the deformation vibrations of O-H from the water and the stretching vibrations of the functional groups C=O (ketone) of fructose and

CH=O (aldehyde) of glucose (Anguebes et al., 2016; Gok et al., 2015).
The absorption band from 1600 cm^{-1} to 700 cm^{-1} is specific to the structure of carbohydrates of honey bee. There are many peaks of the stretching vibrations of bonds C-C, C-C, C-H and the bending vibrations of C-H which are present in chemical structure of carbohydrates (Anguebes et al., 2016; Gok et al., 2015).

CONCLUSIONS

The values of the main parameters required by legislation (moisture, free acidity, electrical conductivity) for all honey samples were found within the recommended limits.
Pearson coefficients showed strong correlations between some parameters: color with total phenols content and total flavonoids content and between electrical conductivity and ash content.
The total phenolic and flavonoid content depend mainly on type of honey.
FTIR analysis gives qualitative information on honey adulteration and confirms different levels of water and sugar in samples.

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