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Physico-chemical and bioactive properties of different floral origin honeys from Romania

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ABSTRACT

In this study, we investigated and compared the physico-chemical properties (moisture, colour, ash, and sugars content) as well as total phenols, total flavonoids and antioxidant activity of several honey samples (24) collected from different regions of Romania. The physico-chemical values were in the range of approved limits (conforming to EU legislation); excepting the monosaccharide values for one sample (T2). For this sample, the other values were within legislation limits. The results obtained showed that the most valuable honey is the honeydew one. Correlation between RSA and total phenols and total flavonoids, respectively, was determined, and a positive correlation was found. This study demonstrates remarkable variation in antioxidant properties and content of total phenols in honey, depending on its botanic or geographic source.

1. Introduction

The quality of Romanian honey available on the market differs on account of various factors like geographical, seasonal and processing conditions, floral source, packaging and storage conditions (Anklam, 1998; Azeredo, Azeredo, de Souza, & Dutra, 2003). For consumers, the sensorial characteristics of honey represent a major parameter in determining the quality of honey, and the parameters with the biggest impact are the colour and the crystallisation state (Moise, Mărghitaş, Dezmirean, & Laslo, 2007). Therefore, new European regulations regarding the quality criteria for honey have been adopted (EEC, 110/2001).

Honey is a supersaturated solution of sugars, which contains more than 180 other constituents like enzymes, amino acids and organic acids, carotenoids, Maillard reaction products, vitamins, minerals and polyphenols (Gheldorf, Wang, & Engeseth, 2002; White, 1979). The minor compounds (Al-Mamary, Al-Meeri, & Al-Habori, 2002) give the bioactive properties of honey, such as phenols (flavonoids and phenolic acids) (Beretta, Granata, Ferrero, Orioli, & Maffei Facino, 2005), and some studies have indicated that these are more potent regarding the antioxidant effect than vitamin C or E (Cao, Sofic, & Prior, 1997). Honey is known to be rich in enzymatic and non-enzymatic antioxidants, including glucose-oxidase, catalase, flavonoids, ascorbic acid, phenolic acids and carotenoids (Aljadi & Kamaruddin, 2004; Batrušaitytė, Venskutonis, & Čeksterytė, 2007). The composition and antioxidant activity of honey depend on the floral source, environmental factors and processing; some reports showed possible correlations between floral origin and flavonoid profiles (Al-Mamary et al., 2002; Yao et al., 2004).

Generally, higher antioxidant activity (expressed as radical scavenging activity) is found in darker honey samples (Beretta et al., 2005) and the variations in the antioxidant activities of honeys are due to the quantitative and qualitative nature of their phenolic contents (Aljadi & Kamaruddin, 2004; Hirano, Sasamoto, Matsumoto, Takura, et al., 2001).

Since the 1970s researchers from different scientific fields have investigated the chemical and biological properties of honey, but only recently has there been an increased interest in application of antioxidants to the medical treatment of different diseases caused by oxidative stress (Aljadi & Kamaruddin, 2004; Beretta et al., 2005; Storz & Imlay, 1999; Zheng & Wang, 2001).

It is expected that honey properties from different botanical sources and location are different. Traditionally, in Romania honey has been used as a sweetener or preservative in food (Mărghitaş, 2005) and in folk medicine all over the world as treatment for a variety of diseases (Meda, Lamien, Romito, Millago, & Nacoulma, 2005; Nagai, Inoue, Kanamori N, & Nagashima, 2006; Orhan et al., 2003). Since, Romanian honey has not been yet studied regarding the total phenols, flavonoid profile and antioxidant activity, our major purpose was to establish these parameters which make it so valuable for consumers.

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2. Material and methods

2.1. Honey sample

Twenty four honey samples: acacia (*Robinia pseudoacacia*) (S), sun-flowers (*Helianthus annuus*) (F), lime (*Tilia sp.*) (T) and honeydew (mountain multi-flora)(M) were harvested from 2005 to 2006 directly from beekeepers and stored at 4 °C, in dark conditions. All honeys were analyzed to determine the following physico-chemical characteristics: moisture, colour, ash, sugars and to measure the phenols and total flavonoids as well as their antioxidant activity.

All analyses were performed in triplicate and according to the methods proposed by the International Honey Commission (Bogdanov, Martin, & Lullmann, 1997) and in agreement with the European Union.

2.2. Chemicals

Ultra pure water, methanol and acetonitrile (HPLC grade) were purchased from Sigma Chemical Co., saccharide standards (fructose, glucose, saccharose, melezitose, maltose and trehalose); $AlCl_3$, NaOH, Na_2CO_3, gallic acid and $NaNO_2$ were purchased from Merck (Germany). 2,2-Diphenyl-1-hydrazyl-hydrate p.a. (DPPH) was obtained from Sigma Aldrich.

All chemicals and reagents used were of analytical grade.

2.3. Apparatus

The following apparatus were used: Abbe digital refractometer-Optic Ivymen System for moisture content, Lovibond Colorimeter PFX 195/7- Tintometer for colour index and gravimetric method was used for ash quantification. An HPLC Shimadzu with IR detector RID-10A and an Alltima Amino 100A column containing amino modified silica gel (250 mm × 4.6 mm, 5 μm) was used for fructose, glucose, saccharose, melezitose, maltose and trehalose determination. Spectophotometric measurements were done with a computer-aided UV-VIS Spectrophotometer 1700 (Shimadzu, Japan) using quartz cuvettes of 1 cm path length.

2.4. Physico-chemical properties

The water, colour and ash content were determined using methods adopted by the International Honey Commission (Bogdanov et al., 1997).

For sugar determination a method described by Bonta, Mărghitaş, Bobiş, and Dezmirean (2007) was used: 5 g of honey were dissolved in water and transferred quantitatively into a 100 ml volumetric flask, containing 25 ml methanol and filled up to the volume with water. The solution was filtered through a 0.45 μm Technochroma syringe filter, collected in vials and stored at 4 °C until further analysis. The mobile phase was acetonitrile/water (75:25 v/v) at a flow rate of 1.3 ml/min and it was filtered through membrane filter (0.45 μm) from Technochroma prior to elution. The injection volume was 10 μl.

A calibration curve was made for each sugar using standard solutions of different concentrations (0.5–80 mg/ml). The linear regression factor of the calibration curves was higher than 0.9982 for all sugars. Sugars were quantified by comparison of the peak area obtained with those of standard sugars. The results for each sugar were expressed as g/100 g honey.

The honey samples in a crystallised state were liquefied in water bath at 40 °C.

2.5. Estimation of total phenols

The total phenol content was determined by a modification of the Folin–Ciocalteu method and the results are expressed as mg gallic acid equivalents (GAE)/100 g. The method (Singleton & Rossi, 1965) modified for honey was developed as follows: 5 g of honey were treated with 50 ml of distilled water, mixed and filtered using a qualitative filter. Five hundred microlitres of this solution was mixed with 2.5 ml Folin–Ciocalteu reagent (0.2 N) for 5 min and then 2 ml of a Na_2CO_3 solution were added (75 g/l). All samples were incubated at room temperature in the dark conditions for 2 h, and their absorbance was read at 760 nm. The blank solution contained methanol instead of honey. For calibration curve, a stock solution of gallic acid (1 mg/ml) was prepared for further dilutions. The linearity obtained was 0.9942 (R^2).

2.6. Estimation of total flavonoids

For the total flavonoid determination, a method described by Kim, Jeong, and Lee (2003) and modified by Blasa et al. (2005) for honey sample was used. Different concentrations of quercetin (5–114 μg/ml) were used for calibration and the linearity was 0.9953 (R^2).Briefly, 1 ml of honey solution (1 mg/ml) was mixed with 0.3 ml $NaNO_2$ 5%, and after 5 min 0.3 ml $AlCl_3$ 10% were added. The honey samples were mixed and six minutes later, were neutralised with 2 ml NaOH solution (1 M). The absorbance was read for all samples at 510 nm and the quantification was done using the calibration curve.

The results were expressed in mg quercetin equivalents (QE) / 100 g of honey, as the average of 3 replications.

2.7. Antioxidant activity

The antioxidant activity (expressed like RSA) was determined for all samples using the method described by Chen, Mehta, Berenbaum, Zangeri, and Engeseth (2000) and modified by Meda et al. (2005) based on DPPH (2,2-diphenyl-1-hydrazyl-hydrate) inhibition. Methanol (HPLC grade) was used for honey sample preparation (0.0065–50 mg/ml). Caffeic acid (0.0065 mg/ml methanol) and vitamin E (0.0065 mg/ml methanol) were used as positive controls. The discoloration grade of 2 ml DPPH (0.02 mg/ml in methanol) with 1 ml honey solution (12.5 mg/ml) was recorded at 515 nm after 15 min.

3. Results and discussion

3.1. Physico-chemical properties

The obtained data are presented in Table 1 and show a good quality of studied honey samples. Values between 15.40% and 20.00% were obtained and they are included in the water range limits approved by the European Commission (EEC, 110/2001). Other authors found for this characteristic values between 19% and 19.7% for Anatolian monofloral honey (Küçük et al.2007). The water content is a good criterion to establish the quality of honey; a higher content can produce honey fermentation during storage.

Normally, there is a positive correlation between the colour, ash content and electrical conductivity of honey. The Romanian blossom honey has a lower ash content than honeydew. Also, the colour index on the Pfund scale and the electrical conductivity is lower (Mărghitaş, 2005).

Sugars represent the main components of any type of honey. Reducing sugars (invert sugar), mainly fructose and glucose, have been found to be the major constituents of honey (Küçük

Table 1
Physico-chemical parameters (crystallization state, water, colour, ash content and the main sugars) of analyzed honey samples

Sample code	Crystallization state	Water (%)	Colour (mm/ Pfund scale)	Ash content (%/ 100 g)	Sugars (g/100 g honey)						
					Fructose	Glucose	F/G	Sucrose	Maltose	Trehalose	Melezitose
S1	Fluid	17.90 ± 0.16	18	0.03 ± 0.01	45.54 ± 0.03	37.05 ± 0.02	1.23	uq	2.06	uq	0.00
S2	Fluid	19.80 ± 0.12	30	0.11 ± 0.01	45.08 ± 0.04	31.99 ± 0.01	1.41	uq	3.57	0.87	0.00
S3	Fluid	17.40 ± 0.12	45	0.28 ± 0.00	43.09 ± 0.01	32.28 ± 0.02	1.34	uq	4.15	uq	0.00
S4	Fluid	17.60 ± 0.12	11	0.08 ± 0.01	45.79 ± 0.02	33.57 ± 0.02	1.36	0.07	2.73	0.50	0.00
S5	Fluid	16.60 ± 0.23	40	0.27 ± 0.01	44.50 ± 0.02	35.63 ± 0.04	1.25	0.36	3.89	uq	0.00
S6	Fluid	18.90 ± 0.24	31	0.12 ± 0.01	39.78 ± 0.04	28.79 ± 0.03	1.38	0.09	4.35	uq	0.00
S7	Fluid	17.00 ± 0.13	32	0.12 ± 0.00	43.43 ± 0.02	31.19 ± 0.03	1.39	uq	4.02	0.80	0.00
S8	Fluid	17.10 ± 0.09	28	0.10 ± 0.01	33.23 ± 0.03	25.26 ± 0.01	1.32	0.06	3.63	uq	0.00
S9	Fluid	17.90 ± 0.12	26	0.09 ± 0.01	44.51 ± 0.02	32.73 ± 0.04	1.36	0.20	3.73	uq	0.00
S10	Fluid	17.80 ± 0.08	22	0.08 ± 0.00	37.31 ± 0.01	28.58 ± 0.03	1.31	0.15	3.22	0.91	0.00
F1	Crystallized	17.80 ± 0.06	83	0.40 ± 0.01	38.37 ± 0.01	42.19 ± 0.02	0.91	0.49	1.45	0.51	0.00
F2	Fluid	19.50 ± 0.06	80	0.35 ± 0.01	44.85 ± 0.05	28.63 ± 0.01	1.57	0.24	2.17	uq	0.00
F3	Crystallized	19.70 ± 0.12	79	0.35 ± 0.00	36.72 ± 0.02	45.57 ± 0.02	0.81	0.34	0.77	0.00	0.00
T1	Fluid	16.70 ± 0.15	41	0.27 ± 0.01	38.75 ± 0.05	36.08 ± 0.03	1.07	uq	5.06	uq	0.00
T2	Crystallized	16.80 ± 0.01	46	0.29 ± 0.01	20.84 ± 0.02	21.65 ± 0.04	0.96	uq	3.93	uq	0.00
T3	Fluid	19.10 ± 0.03	54	0.30 ± 0.01	39.00 ± 0.10	32.96 ± 0.02	1.18	0.23	1.84	uq	0.00
T4	Fluid	17.60 ± 0.04	36	0.19 ± 0.01	36.20 ± 0.20	34.87 ± 0.02	1.04	0.81	2.76	uq	0.00
M1	Fluid	16.60 ± 0.05	96	1.20 ± 0.02	42.58 ± 0.02	38.51 ± 0.02	1.11	0.18	3.15	1.19	2.69
M2	Fluid	15.40 ± 0.06	92	1.19 ± 0.01	37.12 ± 0.02	36.10 ± 0.01	1.03	0.10	3.88	2.82	0.60
M3	Fluid	16.80 ± 0.19	94	1.17 ± 0.01	37.35 ± 0.06	36.02 ± 0.03	1.04	0.15	4.07	3.20	0.32
M4	Fluid	17.90 ± 0.04	94	0.14 ± 0.01	41.38 ± 0.08	38.41 ± 0.02	1.08	0.35	uq	0.87	2.75
M5	Fluid	20.00 ± 0.12	103	1.23 ± 0.01	35.24 ± 0.01	35.40 ± 0.02	1.00	0.17	5.61	1.26	0.51
M6	Fluid	17.40 ± 0.11	98	1.20 ± 0.01	37.27 ± 0.01	32.15 ± 0.03	1.16	uq	3.41	uq	0.01
M7	Fluid	17.60 ± 0.14	96	1.20 ± 0.01	40.09 ± 0.02	35.84 ± 0.03	1.12	0.15	3.01	1.20	2.49

uq- unquantified.
Data are means ± SD of triplicate measurements.

et al., 2007). The blossom honey taken for study presented all tested sugars, excepting melezitose, which is a marker for honeydew (Weston & Brocklebank, 1999). It was expected that the honeydew samples contain melezitose. The registered values for this sugar were between 0.01 g/100g and 2.75 g/100g of honey. The total content of glucose and fructose is over 60 g/100 g of honey, in accordance with the EC Directive 110/2001, for all samples excepting the T2 sample. The fructose–glucose ratio was calculated for all samples. This ratio gives information about the crystallisation state of honey: when fructose is higher that glucose the honey is fluid. This result was confirmed by the crystallisation state of the mentioned honey sample.

3.2. Estimation of total phenols

The concentration and type of phenolic substances depend on the floral origin of honey and those are the major factors responsible for biological activities of honey (Al-Mamary et al., 2002; Küçük et al. 2007; Wei & Zhirong, 2003). The method used was sensitive enough for total phenol estimation in honey. The results are presented in Fig. 1 and the values represent the mean ± SD of three determinations.

From floral honey, the total phenolic substances were higher in sunflower (F) samples, but the honeydew one presented a much higher quantity. The values obtained for Romanian monofloral honey were smaller that those obtained by other authors (Al-Mamary et al., 2002; Aljadi & Kamaruddin, 2004; Beretta et al., 2005) when analysing different origin honey. For honeydew similar values were obtained (113.05 mg ± 1.10) by Meda et al., 2005. Even when the results are expressed in mg catechin/100 g honey the values reported for floral honey were higher (Küçük et al., 2007).

3.3. Estimation of total flavonoids

The method used described previously gives good results for honey and for other matrices also (e.g. mulberry) (Zhishen, Mengcheng, & Jianming, 1999). Using the calibration curve generated by quercetin (R^2 = 0.9953), the total flavonoid content

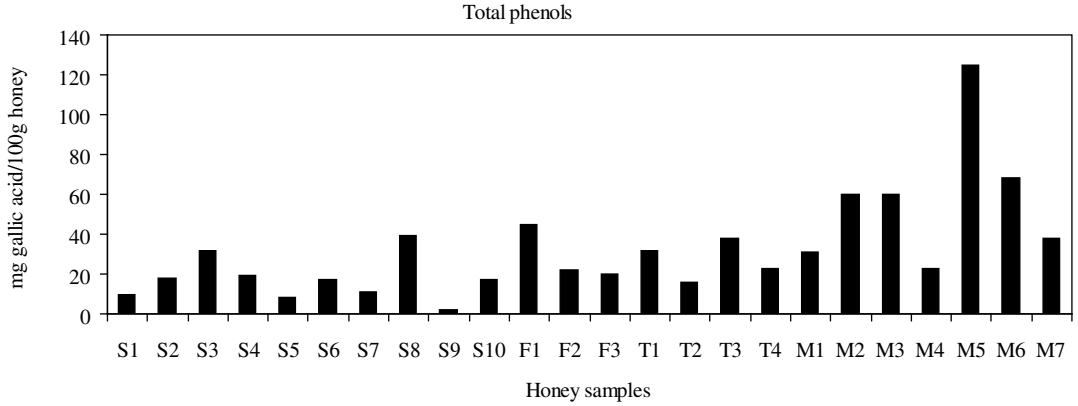

Fig. 1. Graph of total phenols of 24 analyzed honey samples (S-acacia honey; T-lime honey; F-sun-flower honey, M-honeydew), expressed as mg Gallic acid/100 g of honey.

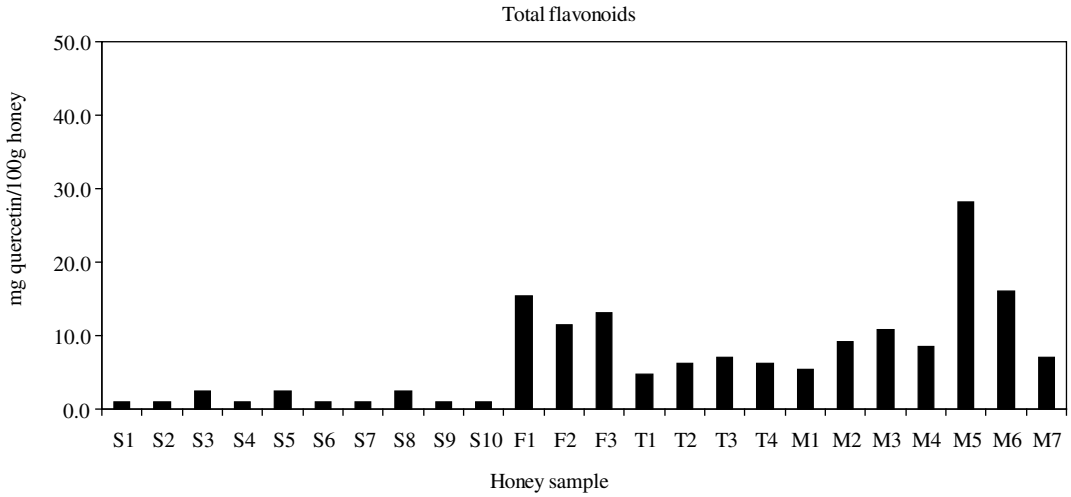

Fig. 2. Graph of total flavonoids of 24 analyzed honey samples (S-acacia honey; T-lime honey; F-sun-flower honey, M-honeydew), expressed as mg Quercetin/100 g of honey.

Table 2
The minimum and maximum values of total phenols, total flavonoids and RSA for every honey type

Sample/ Code	Total phenols (mg gallic acid/100 g)	Total flavonoids (mg quercetin equivalents/100 g)	RSA (% Inhibition)
Acacia/S	2.00–39.00	0.91–2.42	35.80–45.27
Lime/T	16.00–38.00	4.70–6.98	36.60–40.91
Sun-flower/F	20.00–45.00	11.53–15.33	40.65–49.19
Honeydew/ M	23.00–125.00	5.46–28.25	40.67–64.83

Data presents the minimum and maximum values obtained for every honey type.

of honey samples (mg quercetin/100 g honey) ranged between 0.91–2.42 ± 0.02 mg in *Acacia* honey, 4.70–6.98 ± 0.01 mg in *Tilia* honey, 11.53–15.33 ± 0.09 mg in sunflower honey, and 5.46–28.25 ± 0.03 mg in honeydew. The obtained values for all samples are presented in Fig. 2.

For *Acacia* honey the concentrations were under the values given by Meda et al. (2005) (6.14 mg ± 0.35), but for honeydew we obtained higher concentrations (Table 2). The differences are explained by the different floral sources (Amiot, Aubert, Gonnet, & Tacchini, 1989).

A correlation between total phenols and total flavonoids was done using the function CORREL from Microsoft Excel software. The correlation coefficient was 0.84, but other authors maintain that there is not a positive correlation between these two characteristics (Meda et al., 2005).

3.4. Antioxidant activity (RSA)

Generally, the values obtained for Romanian honey were smaller than those obtained by Batrušaitytė et al. (2007) for Lithuanian honey. The maximum value of RSA was obtained for honeydew honey (Fig. 3) and the inhibition percentage for analysed honey samples are presented in Table 2.

The correlation between honey RSA and total phenols, as well as between RSA and total flavonoids was calculated using spreadsheet software (Excel®). A significant correlation ($R^2 = 0.94$) was found between RSA and total phenol content, in accordance with data provided by Beretta et al. (2005), who found a correlation of 0.918 between DPPH and phenol content and data provided by Aljadi and Kamaruddin, 2004, who found a $R^2 = 0.75$. For the correlation between RSA and total flavonoids an $R^2 = 0.83$ was found.

4. Conclusion

In conclusion, several physico-chemical and bioactive properties were studied for 24 honey samples from different geographical areas of Romania. This study showed that the 24 samples of Roma-

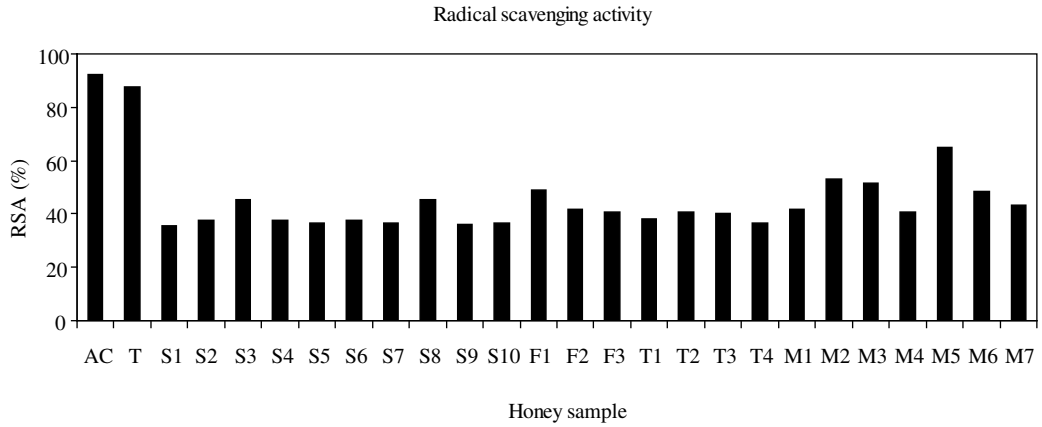

Fig. 3. Antioxidant activity expressed as RSA (%) of analyzed honey samples (S-acacia honey; T-lime honey; F-sun-flower honey, M-honeydew).

nian honey contain phenolic compounds, flavonoids and have a good antioxidant activity, and their content presents variable values for the same type of honey. The richest honey in total phenols and total flavonoids is honeydew honey. It presents the highest percent of inhibition (RSA) and it is followed by sun-flower, lime and acacia honey. The correlation between RSA and total phenols was higher than that between RSA and total flavonoids. Further studies will take in consideration other honeys of different geographic and botanical origin in order to complete the Romanian honey characterisation.

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