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PHYSICO-CHEMICAL PARAMETERS OF ROMANIAN RASPBERRY HONEY

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Abstract: *Six samples of raspberry honey collected from different regions of Romania were analysed in order to confirm that they are classified as monofloral honey. Melissopalynological analysis, alongside the determination of physico-chemical parameters - moisture, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF) content, color, total polyphenols content, flavonoids content, DPPH radical scavenging activity - were chosen as methods of analysis that give indications regarding the botanical origin of honey.The results of the melissopalynological analysis showed that all honey samples had a percentage of Rubus idaeus pollen grains above the minimum of 45%, which was required in order to classify the samples as monofloral honey. The values determined for pH (4.01-4.31), free acidy (20.1-42.1 meq/kg) and electrical conductivity (0.36-0.52 mS/cm) confirmed that the samples were of pure raspberry honey. In the case of moisture content, one honey sample exceeded the moisture content set by Codex Alimentarius (20%). The limit set for HMF content was not exceeded by any raspberry honey sample, for which values of 6.13-26.79 mg HMF/kg were determined. Raspberry honey had high total polyphenols content (8.11-12.86 GAE/100 g), flavonoids content (25.36-41.35 mg QE/100 g) and DPPH radical scavenging activity (58.1-94.35%). These results contribute to the knowledge of the chemical compositions and physical parameters of Romanian honeys.*

Keywords: *honey, authentication, physico-chemical properties*

1. Introduction

Honey is a natural produce widely consumed for its taste and nutritional value, but also for its health benefits [1]. Honey is one of the few natural foods offered today. Clinical studies on honey have shown that it has a broad spectrum of bioactive activity such as anti-carcinogenic, anti-inflammatory, antithrombotic, and analgesic; natural honey consumption also contributes to a reduced risk of cardiovascular disease, being associated with a decrease in body weight [2].

Romania is a country with an old tradition in beekeeping. The National Institute of Statistics reported that honey production in Romania is about 18000 tonnes per year, of which 85% is exported [3]. In Romania, climatic conditions and honey bees are favourable for beekeeping, which presents a real advantage. Since 1961, Romania has been present in international honey production statistics, the percentage of contribution to European honey production ranged from 6.6% (in 1961) to 13.5% (1977) [4]. According to the FAO Stat data, the contribution of Romania to the production of honey in Europe in the year 2017 was of 24611 tonnes, which represents about 6.36% of the total European production. Variations in honey production are closely linked to the climatic conditions and vegetative cycles of the plants that the bees feed on. However, despite these variations Romania and Bulgaria together with Italy and Spain remain important suppliers of organic honey. In developed European countries,

intensive use of pesticides has led to the premature death of hundreds of thousands of bees, and consequently decreased production [4].

In Romania, there are not many types of monofloral honey, and of the produced honey types the most predominant ones are acacia honey (*Robinia pseudoacacia*), tilia (*Tilia* sp.), rape (*Brassica napus* var. *Oleifera*) and sunflower (*Helianthus annuus*). Other types of honey are also found, but these originate from plants with a relatively small geographical area such as raspberry honey (*Rubus idaeus*), mint honey (*Mentha spp*.) or thyme honey (*Thymus serpyllum*).The botanical origin of honey has a great influence on honey quality. The composition of honey depends on the floral source used by bees to collect the nectar, the seasonal and environmental factors, as well as processing [5].

The botanical source of honey is closely linked to its price and, therefore, in order to increase the profit, the producers tend to adulterate it. Equally important is the determination of the geographical origin, which is an important parameter in terms of honey differentiation and commercial value. Depending on the geographical origin, the environment and the area where the hives are located, honey can acquire different characteristics and properties [1] .

The aim of this study was to analyze the physicochemical parameters (melissopalynological analysis, color, pH, free acidity, electrical conductivity, moisture, hydroxymethyl furfural content, total polyphenol content, flavonoids content, and antioxidant activity) of raspberry honey in order characterize it and confirm that is monofloral honey.

2. Materials and methods

2.1. Samples

Six samples of raspberry honey from 2017 and 2018 were purchased from Romanian beekeepers. All samples were liquefied at 50°C and homogenized to carry out the analysis.

2.2. Melissopalynological analysis

10 grams of honey were mixed with 40 mL of distilled water, and the resulting mixture was centrifuged at 4500 rpm for 15 min. after carefully removing the supernatant, the residue was re-dissolved and centrifuged for other 15 min. The sediment was spread on a microscopic slide and the pollen granules were counted by a light microscopy (Motic x 40) [6], [7].

Physicochemical analysis

The physico-chemical analyses were performed according to the analytical methods harmonized by the International Honey Commission [8]. Honey samples were analyzed to determine 9 physico-chemical characteristics: moisture (Abbe refractometer, Leica Mark II Plus), electrical conductivity (portable conduct meter HQ14d, HACH, USA), pH (pH meter METTLER TOLEDO FiveGo, Mettler Toledo, SUA), free acidity (TITROLINE easy, Schott Instruments, Germany), color (photometer Pfund, Hanna Instruments HI 96785 and a portable chromameter, CR-400, Konica Minolta, Japan), hydroxymethyl furfural (HMF) content (Spectrophotometer UV-VIS-NIR SCHIMADZU UV-3600, Schimadzu Corporation, Japan,) total polyphenols content (UV-NIR Spectrometer Ocean Optics HR4000CG-UV-NIR, SUA), flavonoids content (UV-NIR Spectrometer Ocean Optics HR4000CG-UV-NIR , SUA) and DPPH radical scavenging activity.

Moisture

The moisture content was analyzed by refractometry using an Abbe refractometer (Leica Mark II Plus). In order to measure the moisture content the samples were initially liquefied at a temperature of 50ºC.

The refractometer was regularly calibrated with distilled water and the measurement was performed at a temperature of 20ºC. Water content (%) was then obtained from the Chataway table [9] .

pH

The pH was measured on a 10% honey solution with a METTLER TOLEDO FiveGo (Mettler Toledo, SUA) pH-meter.

Free acidity

The free acidity of honey is a measure of the content of all free acids, expressed in milli equivalents/kg honey and was determined by titrimetric method. 10 g honey were dissolved in 75 mL of carbon dioxide-free water, then the pH was measured and the solution was titrated with 0.1M sodium hydroxide solution with TITROLINE easy device (Schott Instruments, Germany) to a pH of 8.30. Calculation was made using the following equation:

Free acidity= mL of 0.1M NaOH x 10(1)

HMF content

The hydroxymethyl furfural (HMF) content was determined using the method proposed by [10]. Honey samples were divided into two clarified aliquots. Water was added to one, and a solution of sodium bisulphite to the other, considered the blank solution. Sodium bisulphite breaks the conjugated double bond responsible of HMF absorbance. The absorbance at λ = 284 nm and λ = 336 nm was then read for the sample solution against the blank solution using a UV-VIS-NIR 3600 spectrophotometer (Schimadzu Corporation, Japan). The results were expressed as mg/kg.

Color

The color was measured with two instruments: a honey color photometer Pfund (Hanna Instruments HI 96785) and a portable chromameter (CR-400, Konica Minolta, Japan).

The CIE L*a*b* coordinates were measured with a portable chromameter (CR-400, Konica Minolta, Japan, where L* is the luminance component (ranging from 0 to 100), while a* and b* are colour coordinates related with the red/green and yellow/blue, respectively.

Electrical conductivity

The portable conduct meter HQ14d (HACH, USA) was used to measure the electrical conductivity of a solution of 20 g dry honey dissolved in 100 mL distilled water. The result was expressed in milliSiemens per centimetre (mS·cm-1)

Determination of total phenolic content

The total content of polyphenols (TPC) was determined using the Folin-Ciocalteu method [11],[12]. The results were expressed as mg gallic acid equivalent (GAE)/100 g of honey [13].

The method proposed by Biesaga et al. [14] was used to determine TPC and sample preparation was made, as follows: 1 g of honey sample were extracted with 5 mL of 40% methanol/acidified water (v/v, pH = 2, adjusted with HCl). Then, the samples were stirred for 15 min with a magnetic stirrer. 0,2 mL of extract were mixed with 2 mL of Folin-Ciocalteu reagent previously diluted in a ratio of 1:10 with distilled water and 1,8 mL of Na_2CO_3 solution (7.5%, w/v). After incubating in the dark for 30 min, the absorbance of all samples were measured at 750 nm with a Ocean Optics HR4000CG-UV-NIR spectrometer (SUA). A calibration curve prepared with gallic acid (0-1000 mg/L) was used.

Flavonoids

5 mL of extract obtained according to the method proposed by Biesaga et al. [14] was mixed with 300 µL of $NaNO_2$ 5% and

300 µL of $AlCl_3$. After 5 minutes in the dark the samples mix with 2 mL of NaOH 1N and after another 6 minutes in the dark, the absorbance of all samples was measured at 510 nm with an Ocean Optics HR4000CG-UV-NIR spectrometer (SUA).

DPPH assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of honey samples was determined as described by Brand-Williams et al. [15]. Sample preparation was made, as follows: 1 g of honey sample was dissolved in 5 mL of methanol 40% (v/v, with acidified water) and filtered. The absorbance of the solution was measured at 515 nm using a Ocean Optics QE65000 spectrometer (SUA). The results were expressed as % DDPH using the formula:

$$\% \text{ DPPH} = \left(A_0 - \frac{A_1}{A_0}\right) \times 100 \quad (2)$$

Where: A_0= The DPPH absorbance,
A_1= The sample absorbance

3. Results and discussion

Pollen analysis

Pollen analysis is a method developed and proposed by the International Bee Botanical Commission (IBBC) in 1970 and revised in 1978 [16]. This method consists of identifying the pollen granules by microscopic analysis in order to determine the plants visited by bees during honey production [17]. Honey is classified as monofloral honey when at least 45% of the pollen grains belong to a single plant species and this category of honey is the most preferred by consumers for its specific aroma, taste and biological properties [18], [19].

The *Rubus idaeus* pollen was predominant in all samples, accounting for more than 45% pollen grains. Therefore, the studied samples correspond to monofloral *Rubus* honeys. Escuredo et al. [20] reported a variation in the percentage of *Rubus* pollen from 46.4 to 91.3% when they studied thirty-three honey samples that were collected from Galicia (Northwest Spain).

Moisture content

According to Codex Alimentarius [25] moisture content should be lower than 20%. Honey samples that do not meet this criterion may be unstable during storage [21], the moisture content of honey being a very important factor in determining quality and stability [22]. The moisture content is largely related to the harvest, season and the maturity of the honey in the hive [23]. During storage, low moisture may lead to the development of caramelization and the Maillard reaction, while higher water content may cause honey to ferment and acetic acid to form [24]. The moisture content of the analyzed samples ranged from 17.32 % to 20.12%. The results were in accordance with the values reported by Sohaimy et al. [22] when analyzing honey from Egypt. When analyzing acacia and tilia honey, Oroian et al. [3] reported similar results for moisture content. Sakač et al. [21] reported a moisture content in the range of 13.2–21.3% and 10.2–24.1% for acacia honey and sunflower respectively, which means that some samples exceeded the limit (>20%) [25].

pH

The pH of the raspberry honey samples ranged from 4.01 to 4.31. The values were similar to those reported by White [26], who reported pH values that ranged from 3.2 to 4.50. Escuredo et al. (2013) [27] reported pH values between 3.50 and 5 for honey from north-western Spain, while Terrab et al. [28] reported pH values between 3.56 and 4.79 in a study of Spanish thyme honey [23]. The pH values are of particular importance during the extraction and storage of honey as they influence the texture, stability and storage

time; the average value reported by Terrab et al. [28] was 4.2.

Free acidity

The free acidity of honey depends on the organic acids produced from nectar during the ripening by glucose oxidase. Geographical origin and harvest season are some factors that influence total acidity [29]. The free acidity of raspberry honey analyzed in this study ranged from 20.1 to 42.1 meq/kg. Moujanni et al. [30] reported in their study that free acidity showed values between 14.07 and 32.29 meq/kg, and all the samples are below the legal acidity limit, which states no more than 50 meq/kg for the free acidity [31]. Sakača et al. [21] reported in a previous study that the honey with the lowest acidity was acacia honey, with an average value of 9.77 ± 2.08 meq/kg, while honeydew was characterized by a higher mean value (19.3 ± 1.88 meq/kg), which does not differ from sunflower honey (19.1 ± 3.19 meq/kg).

HMF content

HMF is a parameter that indicates the degree of freshness of honey and consequently its degree of deterioration. The causes of honey deterioration could be the strong or prolonged thermal treatment and the inadequate storage conditions [32]. The maximum level of HMF allowed in honey is 40 mg/kg [25] and the raspberry honey samples do not exceed the HMF limit (6.13-26.79 mg HMF/kg).

Color

Honey color is one of the most important sensory parameters for consumers. The color of honey depends on the type of honey and certain chemical characteristics such as mineral content [33].The transition elements existing in honey may form complexes with organic honey compounds that tend to be highly colored and thus affect the color of the honey [34]. Changes in honey color appear slowly during storage or rapidly during heat treatment as a result of the Maillard reaction [35].

Table 1

Physical-chemical parameters of raspberry honey

Raspberry Honey							
Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	F -ratio
L*	35.41bc	38.77a	36.4b	34cd	33.16d	28.86e	50***
h^*_{ab}	64.42d	74.94b	65.19d	69.73c	83.67a	82.71a	315***
C^*_{ab}	23.65bc	25.44a	24.93ab	20.64d	25.03ab	23.18c	14**
Color (mm Pfund)	73.5a	64c	74.5a	68.5b	39e	49d	913***
pH	4.04a	4.31a	4.21a	4.29a	4.01a	4.1a	0.79ns
Free acidity (meq/kg)	23.7c	24.3c	20.1d	25.4c	42.1a	28.2b	144***
Electrical conductivity (mS/cm)	0.367c	0.524a	0.382c	0.427b	0.449b	0.528a	41***
Moisture (%)	17.32c	17.44c	18.2bc	17.92c	20.12a	18.92ab	9**
HMF (mg/kg)	21.4c	6.58d	26.79b	4.94d	46.4a	6.13d	533***
Total phenolic content (mg GAE/ 100 g)	24.58a	25.72a	22.32b	14.48e	16.22d	16.4c	122***
Flavonoids content (mg QE/100 g)	25.36c	35.29b	41.35a	39.54a	26.29c	33.69b	98***
DPPH	81.99b	94.35a	75.77c	92.13a	58.1e	71.99d	165***

The color of raspberry honey ranged from extra light amber (39 mm Pfund) to light amber (74.5 mm Pfund). Escuredo et al. [20] reported that according to the obtained results, it seems that *Rubus* honey is characterized by an amber color. The honey samples analyzed in their study had an amber color (mean value of 94 mm Pfund), although they varied from light amber (39 mm deep) to dark amber (150 mm deep). The color of honey depends on various factors, but mainly on its floral origin [36]. All honey samples showed similar lightness values (26.52-38.77).

Electrical conductivity

Electrical conductivity is an important physical parameter used to authenticate unifloral honey types. Electrical conductivity is a factor integrated in the new international standards regarding the differentiation between honeydew and flower honey; the specific electrical conductivity ranges for mixed honey between 0.5-0.8 mS/cm, while a value lower than 0.5 mS/cm indicates pure floral honey, with numerous exceptions [37].A value greater than 0.80 mS/cm is not acceptable for a floral honey because this is specific to honeydew [3]. In this study, 2 samples had values higher than 0.5 mS/cm, while the others had an electrical conductivity between 0.36 and 0.45 mS/cm, which confirm that the samples are pure monofloral honey. Kaskoniene et al. [38] confirmed that floral honey has a lower electrical conductivity than that of honeydew and thus this parameter represents a quality indicator that serves as a mean to distinguish between honeydew and floral honey [3]. The value of the electrical conductivity increases with the increase of the ash and acid content of honey [39].

Total phenolic content

The functional properties of honey are related to the amount of natural antioxidants from pollen collected by bees and other floral nectars [40]. The constituents of honey that are responsible for the antioxidant effects are: flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, products resulting from the Maillard reaction [41], hydroxycarnic acids, and anthocyanins [42]. Phenolic acids and flavonoids have been extensively investigated in honey [43] and are used to evaluate honey quality. The antioxidant activity is correlated with the total concentration of phenols, confirmed for seven types of honey that come from Italy [44] and for four types from Romania [36]. In another study on Portuguese honey it was shown that polyphenols in honey are responsible for its antimicrobial effects [45].

The total phenolic content (TPC) of raspberry honeys ranged from 8.11 to 12.86 mg of gallic acid equivalent (GAE)/100 g of honey, the average value being 19.95 mg GAE/100 g.In the case of Irish polifloral honeys TPC ranged from 2.59 to 81.10 mg GAE/100 g of honey (n=124, Mean ± SD=23.84 ± 13.07) [46]. The variation of TPC in different types of honey depends on their floral origin, Kaškonienė et al. [47] considered that heather honey has a higher average TPC (20.12 ± 0.55 mg/100 g) compared to rape honey. The difference in values can be attributed to several factors, including the geographical origin, the "purity" of the honey and the storage conditions [46]. Gül & Pehlivan [48] reported an average total phenolic content (TPC) of honey samples of 185.81 ± 13.01 mg GAE/100 g honey, but this ranged from 470.70 ± 7.43 to 34.37 ± 0.44.The highest phenolic content of samples was found in parsley, rhododendron, carob, and chestnut honey samples as 470.70 ± 7.43, 408.35 ± 4.71,

336.31 ±3.91 and 327.60 ± 0.88 mg GAE/100 g, respectively; and the lowest phenolic content was found in wild mint (34.37 ± 0.44 mg GAE/100g) and acacia (51.91 ± 1.32 mg GAE/100 g).

Flavonoids content

The amount of flavonoids in honey can reach up to 6 mg/kg, while their quantity is much higher in pollen (0.5%) and propolis (10%); according to literature, the most frequent flavonoids found in honey are pinocembrin, apigenin, campferol, quercetin, pinobanksin, luteolin, galangin, hesperetin, and isorhamnetin [13]. A large number of flavonoids, such as pinobanksin, pinocembrin and chrysin, which are found in honey, do not come from the nectar of flowers, but from propolis [49].

In general, the flavonoid content was higher than that of phenolic acids. The flavonide content of raspberry honey varied between from 25.36 to 41.35mg QE/100 g, with an average value of 33.58 mg QE/100 g. Tanleque & Escriche [50] reported that the highest level of flavonoids was identified in samples from Nampula with a value of 30.45 mg/100 g, followed by Sofala (19.54 mg/100 g), Zambezia (15.22 mg/100 g) and Manica (12.95 mg/100 g).

DPPH assay

The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for determination of free radical scavenging activity, usually expressed as IC50, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Raspberry honey from this study has a DPPH value between 58.1 and 94.35%, with an average value of 79%.

Bertoncelj et al. [51] reported results showing that monofloral honey, acacia and lime had lower antioxidant activities, as these samples had higher IC50 values. Their IC50 values were of 53.8 and 28.8 mg/mL, respectively, and were significantly higher ($p < 0.05$) than the IC50 values of other types of honey. Chestnut and multifloral honey had IC50 values from 7.2 to 10.7 mg/mL. For pine honey sample was determined a value of 65.52 mg/mL, while the antioxidant activity of citrus honey sample was 2.01 mg/mL [48]. Noor et al. [52] found similar results (2.85–39.86 mg QEA/100 g) in a variety of honey samples across Pakistan.

4. Conclusion

The physico-chemical characterization of raspberry honey samples collected from different areas of Romania in the years 2017 and 2018 was performed to examine the quality of honey samples and to determine any similarities and differences that may be attributed to the botanical origin of honey. All the investigated honey samples (6 samples) met the quality criteria examined (moisture, pH, free acidity, HMF content, color and electrical conductivity), except for a sample that in the case of moisture content exceeds the allowed limit. The pollen analysis together with the physicochemical parameters indicates that the honey samples analyzed were monofloral raspberry samples. In the raspberry honey an average value of 19.95 mg GAE/100 g was obtained for the total polyphenol content. In terms of flavonoid content, the average value was higher; respectively 33.58 mg QE/100 g. Raspberry honey from this study had high antioxidant activity, with an average value of 79%.

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