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II.6. SUGARS CONTENT AND PHYSICOCHEMICAL PARAMETERS OF ROMANIAN RAPE HONEY

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Abstract

The aim of this study was to evaluate the physicochemical characteristics of rape honey collected from different regions of Romania. Five rape honey samples were selected to determine their physicochemical parameters, sugar content and antioxidant activity, including total polyphenols content and total flavonoids content. The results of the melissopalynological analysis showed that all honey samples have a percentage of pollen grains Brassica napus well above the minimum of 45%, which is necessary to classify the samples as monofloral honey. The results obtained for rape honey samples indicated a low electrical conductivity (118-173 μS/cm), a pH between 4.03-4.24 and a free acidity between 12.37-25.54%. The limit set for HMF content was exceeded by a sample of rape honey (48.6 mg HMF/kg) and the moisture content varied between 17.30-19.12% and was not exceeded the moisture content set by Codex Alimentarius (20%). Fructose, glucose, sucrose, maltose, turanose, trehalose, melezitinose, and raffinose were identified and quantified in all samples. Results were submitted to analysis of variance (ANOVA) using Statgraphics Centurion XVIII software trial version.

Key words*: authentication, honey, physicochemical properties.*

INTRODUCTION

In recent years, rape crops have grown considerably in Romania because the demand for rape oil, which is used for food and biodiesel, and for animal feed has increased. The large number of rape crops has also led to an increase in the production of rape honey.Rape honey has a light amber color, is sourish-sweet, and has a slightly fermented, musty aroma of rape flower (Siegmund et al., 2017; Wang et al., 2014). The aroma and taste of honey are given by the content in volatile etheric oils and by other volatile components existing in honey in very low concentrations. Rape honey has a discreet aroma, but the intensity of the aroma varies depending on the degree of freshness (Mărghitaș, 2005).

Rape honey has high glucose content and crystallizes very quickly with small crystals but this does not adversely affect its quality (Persano Oddo et al., 2004). Rape honey has pollen that contains 24% crude protein and amino acids such as methionine, threonine and valine.

The existing honey varieties have different composition depending on the source of origin (plants and environmental conditions). The properties of honey are thus influenced by the nectar/pollen originating from the plant, moisture content, aroma, color, or sugar content (da Silva et al., 2016). Honey has more sugars in its composition than any other product of animal origin and the sugar content depends largely on the origin of honey (botanical and geographical) and is influenced by processing, storage and climate (Xu et al., 2019; Da Silva et al., 2016).

Honey is a healthy food that is widely consumed both for its sweet taste and its high nutritional and medical importance (Khan, et al., 2018). Honey is renowned for its antimicrobial properties, properties that are attributed to phenolic compounds derived from honey (Estevinho et al., 2008). Studies have shown that honey has a broad spectrum of bioactive activity such as: anti-inflammatory, anti-carcinogenic, analgesic, antithrombotic activity. Natural honey also has the ability to reduce the risk of cardiovascular disease, and honey consumption may also be associated with a decrease in body weight (Yaghoobi et al., 2008). The authenticity of honey implies both aspects of its geographical and botanical origin and the fact that honey must be entirely pure, with no adulteration with any type of sugar syrup (Xu et al., 2019). The demand for monofloral honey has increased considerably in the last years and for this reason it is necessary to be able to determine some

parameters that contribute to the authentication of the origin of honey that include the botanical and geographical source (Da Silva et al., 2016). Honey authentication is possible by combining the determination of physicochemical parameters with the melissopalynological analysis (Oroian et al., 2015). In recent years, new analytical methodologies have been used to determine the botanical origin including chromatographic, molecular and biological spectroscopic methods (Siddiqui et al., 2017; Escriche et al., 2017).

The purpose of this work was to determine for Romanian rape honey the physicochemical parameters (melissopalynological analysis, moisture, color, pH, free acidity, electrical conductivity), hydroxymethylfurfural content, antioxidant activity-DPPH radical scavenging activity, total polyphenols content, total flavonoids content and sugar content.

MATERIALS AND METHODS

Samples

Five samples of rape honey from 2017 and 2018 were purchased from different regions of Romania. The honey samples were liquefied at 50°C and homogenized prior to analysis.

Melissopalynological analysis

A mixture of 10 grams of honey and 40 mL of distilled water was prepared and then centrifuged at 4500 rpm for 15 minutes. The resulting residue was mixed again with water and subjected to centrifugation for another 15 minutes.The sediment collected from centrifugation by removing the supernatant was spread on a microscopic lamella and the pollen grains were counted with a Motic microscope (Motic, China) at ×40 magnification (Louveaux & Vorwohl, 1970).

Physicochemical analysis

The color of the honey samples was analyzed by two methods, namely using a portable chromameter, CR-400 (Konica Minolta, Japan) and a Pfund photometer HI 96785 (Hanna Instrumets, USA).

Using the analytical methods proposed by the International Honey Commission, the physicochemical parameters were determined, as follows: moisture content (using Abbe refractometer, Leica Mark II Plus), pH (using a pH meter Mettler Toledo FiveGo, Mettler Toledo, USA), free acidity (using TITROLINE easy, Schott Instruments, Germany) and electrical conductivity (using a portable conductometer HQ14d, HACH, USA).

The hydroxymethylfurfural (HMF) content was determined using the White method **(**White, 1978) and a UV-VIS-NIR SCHIMADZU UV-3600 spectrophotometer (Schimadzu Corporation, Japan).

To identify the total polyphenols content, total flavonoids content and to determine the antioxidant activity, the preparation of the samples was done according to the method proposed by Biesaga & Pyrzyńska (2013), as follows: 1 g of honey sample was extracted with 5 mL of 40% methanol/acidified water (v/v, pH = 2 adjusted with HCl). The honey samples were homogenized with a magnetic stirrer for 15 min.

To identify the total polyphenols content 0.2 mL of extract was mixed with 2mL of Folin - Ciocalteu 1:10 and 1.8 mL Na_2CO_3, 7.5% (w/v). After incubation in the dark the absorbance of the reaction mixture was measured at 750 nm.

To identify the total flavonoids content 5 mL of extract obtained according to the method proposed by Biesaga & Pyrzyńska (2013) were mixed with 300 µL of $NaNO_2$ 5% (w/v) and 300 µL of $AlCl_3$ (w/v). After 5 minutes in the dark the samples were mixed with 2 mL of NaOH 1 N and after another 6 minutes in the dark the absorbance of all samples was measured at 510 nm.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of honey samples was determined as described by Brand-Williams et al. (1995). 35 µL of honey sample were mixed with 250 µL of DPPH. The absorbance of the solution was measured at 515 nm. The results were expressed as % DPPH using the formula in Eq. 1:

$$\% \text{ DPPH} = \left(A_0 - \frac{A_1}{A_0} \right) \times 100, \quad (1)$$

where A_0 is the DPPH absorbance, A_1 is the sample absorbance.

The UV-NIR HR4000CG-UV-NIR spectrometer (Ocean Optics, USA) and UV-NIR HR4000CG-UV-NIR spectrometer (Ocean Optics, USA) were used to measure the absorbance.

Determination of sugars composition

To determine the sugar content, the honey samples (5 g of each sample) were dissolved in distilled water (40 mL), mixed with 25 mL of methanol (in a 100 mL volumetric flask) and then brought to volume with distilled water. The samples obtained as described above were analyzed with a HPLC instrument (Schimadzu, Kyoto, Japan). This instrument was equipped with a LC-20 AD liquid chromatograph, SIL-20A auto sampler, CTO-20AC column oven, and RID-10A refractive index detector. The separation was performed on a Phenomenex Luna® Omega 3 µm SUGAR 100 Å HPLC Column 150 x 4.6 mm. Before being injected into the HPLC instrument, the samples were filtered through 0.45 µm PTFE membrane filters. The sample volume injection was 10 µL. The flow rate was 1.3 mL/min and the mobile phase was acetonitrile: water (80:20, v/v); the temperature of the column and detector was 30 °C.

RESULTS AND DISCUSSIONS

Melissopalynological analysis

Melissopalynological analysis is the method that has been used over the years to identify the botanical and geographical sources of honey (Soria et al., 2004). Pollen analysis provides valuable information regarding the organoleptic properties of honey and its composition (Von der Ohe et al., 2004). A percentage of 45% pollen granules present in honey samples indicates that honey is authentic and thus its botanical origin is known (Siddiqui et al., 2017). In the rape honey samples analyzed in the present study the percentage of pollen grains had a maximum value of 73% and a minimum of 53%. The results of the melissopalynological analysis indicate that the 5 samples of rape honey were authentic. Rajs et al. (2017) analyzed 21 samples of Croatian rape honey and reported that the percentage of pollen varied between 60 and 98%.

Color

An important feature for the market is the color of honey. Color is the main feature evaluated by consumers as an attribute of quality (Dominguez & Centurion, 2015). Honey color provides information about the quality of honey, the source from which it comes and also about the particularities of production (Tuberoso et al., 2014). The specific color of the rape honey that was analyzed in this study was extra light amber because the values on the Pfund scale varied between 18.81 and 40.09 mm Pfund (Table 1). Wang et al. (2014) studied ten samples of rape honey from Shaanxi, China and reported a color variation ranging from 20.5 to 25.0 mm Pfund.

pH

The organic acids in the composition of honey are responsible for the pH values between 3.5 and 5.5 and offer protection in case of microbial attacks. If the pH value increases above 7.2 then microorganisms have an environment favorable for development, and as a result the pH can be considered an indicator of microbial growth (Da Silva et al., 2016).

The analyzed rape honey samples had a pH value between 4.03 and 4.24. The pH of the rape honey samples investigated by Wang et al. (2014) was between 3.83 and 4.25. Tomczyk et al. (2019) analyzed in their study the rape honey from Poland and Slovakia and reported a pH value of 3.88 (Polish honey) and 3.61 (Slovak honey).

Free acidity

Free acidity is an indicator of the freshness of honey. When honey begins to deteriorate, the process of fermentation of sugars into organic acids appears and the result is the increase of free acidity (Da Silva et al., 2016). The rape honey samples analyzed in this study had a free acidity between 12.37 and 25.54 meq/kg (Table 1). According to the Council Directive (2001) the maximum limit for free acidity is 50 milli-equivalents of acid per 1000 grams (Codex Alimentarius, 2001) and the values of free acidity obtained in this study show that the honey samples analyzed were fresh.

Kędzierska-Matysek et al. (2016) reported for the rape honey collected from Lublin, Poland that all the samples that were analyzed were acidic (the average value was 18.71). Dinkov et al. (2016) reported free acidity values between 35-38 meq/kg when analyzing rape honey from Stara Zagora, Bulgaria.

Table 1. Physicochemical properties for Romanian rape honey. Values and standard deviation in brackets

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F –ratio
L*	41.68(0.60)^{c}	48.63(0.69)^{a}	43.54(0.62)^{b}	36.43(0.52)^{e}	39.29(0.56)^{d}	116.97***
h^*_{ab}	90.83(1.29)^{b}	95.77(1.37)^{a}	83.62(1.19)^{c}	92.87(1.32)^{ab}	96.24(1.37)^{a}	30.16***
C^*_{ab}	24.93(0.35)^{c}	26.82(0.38)^{b}	30.79(0.43)^{a}	18.32(0.26)^{e}	19.22(0.27)^{d}	450.29***
Color (mm Pfund)	27.72(0.39)^{c}	18.81(0.26)^{e}	40.09(0.57)^{a}	36.13(0.51)^{b}	21.78(0.31)^{d}	900.71***
pH	4.19(0.059)^{a}	4.24(0.06)^{a}	4.03(0.057)^{b}	4.10(0.058)^{ab}	4.20(0.06)^{a}	4.03ns
Free acidity (meq/kg)	12.37(0.17)^{e}	16.23(0.23)^{d}	25.54(0.36)^{a}	17.32(0.24)^{c}	18.83(0.26)^{b}	659.19***
EC (µS/cm)	118.89(1.69)^{e}	126.52(1.80)^{d}	173.89(2.48)^{a}	146.66(2.09)^{b}	136.12(1.94)^{c}	223.77***
Moisture (%)	17.82(0.254)^{bc}	19.12(0.273)^{a}	18.41(0.263)^{b}	17.30(0.247)^{c}	18.09(0.258)^{b}	13.73**
HMF (mg/kg)	22.67(0.323)^{b}	12.74(0.18)^{c}	48.60(0.69)^{a}	9.48(0.13)^{d}	7.40(0.10)^{e}	4414.78***
TPC (mg GAE/100 g)	12.75(0.18)^{d}	23.11(0.33)^{c}	26.70(0.38)^{b}	31.04(0.44)^{a}	23.03(0.32)^{c}	771.27***
FC (mg QE/100 g)	7.66(0.10)^{d}	7.87(0.11)^{d}	16.23(0.23)^{a}	13.24(0.18)^{b}	12.41(0.17)^{c}	928.4***
DPPH	50.59(0.72)^{cd}	52.40(0.74)^{bc}	53.01(0.75)^{b}	55.80(0.79)^{a}	49.06(0.70)^{e}	23.45**
Fructose (%)	34.87(0.49)^{b}	37.61(0.53)^{a}	35.64(0.50)^{b}	35.23(0.50)^{b}	35.09(0.50)^{b}	9.49ns
Glucose (%)	33.57(0.47)^{c}	28.25(0.40)^{e}	29.46(0.42)^{d}	36.09(0.51)^{a}	34.39(0.49)^{b}	103.94***
Sucrose (%)	0^{b}	0.62(0.008)^{a}	0^{b}	0^{b}	0^{b}	9801***
Turanose (%)	0.29(0.004)^{bc}	4.16(0.05)^{a}	0.34(0.004)^{b}	0.28(0.004)^{bc}	0.26(0.003)^{d}	8274.44***
Maltose (%)	1.37(0.01)^{c}	5.97(0.08)^{a}	1.66(0.02)^{b}	0.94(0.13)^{d}	0.89(0.01)^{d}	5384.39***
Trehalose (%)	1.49(0.021)^{c}	11.34(0.16)^{a}	1.99(0.02)^{b}	0.99(0.01)^{d}	0.911(0.013)^{d}	7233.83***
Melesitose (%)	0.90(0.01)^{c}	2.95(0.04)^{a}	1.16(0.01)^{b}	0.63(0.009)^{d}	0.60(0.008)^{d}	4016.17***
Raffinose (%)	0.19(0.002)^{b}	0^{e}	0.36(0.005)^{a}	0.11(0.001)^{c}	0.096(0.001)^{d}	4725.12***
F/G ratio	1.02(0.01)^{c}	1.31(0.01)^{a}	1.19(0.01)^{b}	0.96(0.01)^{d}	1.01(0.014)^{c}	174.47***

ns - not significant ($p > 0.05$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $^{a-d}$different letters in the same row indicate significant differences between samples ($p < 0.001$)

Moisture

The moisture content of honey is dependent on the season, harvest and the degree of maturity of honey in the hive (Karabagias et al., 2014). Honey moisture can be considered a limiting factor in terms of quality and stability (El Sohaimy et al., 2015). High moisture content leads to degradation of honey due to the fermentation process, while low moisture content results in the Maillard reaction and the development of caramelization (Chirifie et al., 2006).

In this study, the moisture content ranged between 17.30 and 19.12%, which showed that the rape honey samples were in accordance with the 20% limit imposed by Codex Alimentarius (2001). In the rape honey from Croatia and Slovakia studied by Raj et al. (2017) the measured values for moisture content ranged between 16.4 and 19.4%. Oroian et al. (2018) reported for sunflower, acacia, tilia, honeydew and polyfloral honey from Romania moisture content between 14.5 and 19.8%.

Electrical conductivity

In the studied samples the electrical conductivity varied between 118.89 and 173.89 µS/cm. This parameter was recently introduced in international standards as its value provides information on the botanical origin of honey.

The electrical conductivity with a value < 500 µS/cm indicates pure floral honey, while values between 500-800 µS/cm are specific for mixed honey (Saxena et al., 2010). The electrical conductivity of honey increases directly in relation to two other important parameters, namely the acid content and the ash content (El Sohaimy et al., 2015).

Szczęsna et al. (2011) reported a lower electrical conductivity for rape honey (200 µS/cm) and Tomczyk et al. (2019) reported for the Polish rape honey a similar average value (230 µS/cm). Oroian et al. (2016) reported a low electrical conductivity for acacia honey (156.58 µS/cm) as opposed to sunflower (346.1 µS/cm) and tilia honey (549.31 µS/cm). These results indicate that light honey has a low electrical conductivity.

HMF content

The degree of freshness of honey can be indicated by the content of HMF (Wang et al., 2014). As seen in Table 1, honey samples had HMF content between a minimum of 7.40 mg/kg and a maximum of 48.60 mg/kg. The limit set for HMF content (40 mg/kg) was exceeded by a sample of rape honey. The increased content of HMF can be attributed to an overheating of the honey during processing and/or storage. Wilczyńska (2012) reported for 5-HMF content values between 0.6 and 4 mg/kg. In the study by Raj et al. (2017) none of the rape honey samples from Croatia exceeded the HMF limit allowed by law. Oroian et al. (2015) reported that all analyzed samples were fresh and had a HMF content below 40 mg/kg.

Total phenolic content and total flavonoids content

Honey is a natural product that contains many phenolic compounds, and the amount and nature of phenolic acids and flavonoids are of great interest, as these compounds are known to have different nutritional properties, as well as a potential role in treating different diseases and contributing to human health (Ciulu et al., 2016). The total phenolic content (TPC) of Romanian rape honeys ranged from 12.75 to 31.04 mg of gallic acid equivalent (GAE)/100 g of honey. Tomczyk et al. (2019) reported for rape honey values between 21 mg of gallic acid equivalent (GAE)/100 g of honey for Slovak rape honey and 25 mg of gallic acid equivalent (GAE)/100 g for Polish rape honey. Bonvehi et al. (2019) studied avocado honey and reported values for TPC content between 103 and 137 mg GAE/100 g of honey.

The flavonoids content of rape honey varied from 7.66 to 16.23 mg QE/100 g.

Ibrahimi & Hajdari, (2020) studied 100 honey samples of different botanical origins from Kosovo and determined the highest TPC content in forest honey (84.17 mg GAE/100 g) and the lowest content in acacia honey (25.76 mg GAE / 100 g).

For the same honey samples, Ibrahimi & Hajdari (2020) reported that the lowest flavonoids contents were in acacia honey (1.11 mg CE/100 g), followed by mixed honey (3.44 mg CE/100 g), while the highest flavonoids contents were reported in forest honey samples (7.51 mg CE/100 g).

DPPH assay

The antioxidant activity is directly correlated with the DPPH radical scavenging activity(Ouradi et al, 2020). In the present study the highest DPPH radical scavenging activity (Table 1) was 55.80%. In comparison, in Slovak rape honey the DPPH radical scavenging activity was 11.76% and in the Polish rape honey was 21.21% (Tomczyk et al., 2019). In thyme honey samples from Marocco the DPPH radical scavenging activity was 36.54 % (Ouradi et al, 2020).

Sugars content

Honey is a complex mixture of sugars (80%) and other components (Soares et al., 2017). Cotte et al. (2004) argued that the ratio of specific carbohydrates (fructose and glucose), as well as the amount of sugars are useful tools that can be applied to distinguish monofloral honey. The content and composition of honey sugars are influenced by botanical source, geographical origin, processing and storage conditions and climate (Escuredo et al., 2014). The viscosity, energy value, granulation and hygroscopicity of honey are influenced by the sugar content (Kamal and Klein, 2011).

In the samples of rape honey the variation of fructose content was not significant in comparison to the glucose content, which presented a significant variation (29.46-36.09%). Sucrose was identified in a single sample of rape honey, while the content of turanose was constant in 3 samples but was higher in one of the rape honey samples. All types of honey that crystallize quickly have high glucose content and the F/G ratio is about 1 (Rajs et al., 2017).

Similar results for fructose (36.35-36.39%) and glucose (29.69-32.92%) content in rape honey were reported by Tomczyk et al. (2019). Rajs et al. (2017) reported that the predominant sugars in Croatian rape honey were fructose (38.3) and glucose (36.1), and the average value of fructose/glucose (F/G) ratio was 1.1.

CONCLUSIONS

In this study were analyzed the content of pollen, the physicochemical parameters, and the sugar composition of Romanian rape honey in order to classify it as monofloral honey. The results obtained for the targeted parameters allowed the characterization of honey in terms of physicochemical properties. The high percentage of pollen grains of Brassica napus, together with the physicochemical parameters confirm that the honey samples analyzed were samples of monofloral rape honey. The values determined for the physicochemical parameters of the rape honey were in accordance with the European standars for honey.

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