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ORGANIC ACIDS AND PHYSICO-CHEMICAL PARAMETERS OF ROMANIAN SUNFLOWER HONEY

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Abstract: *The samples of sunflower honey collected from Romania were analyzed in order to characterize them from the physico-chemical point of view. Melissopalynological analysis, together with the determination of organic acids and physico-chemical parameters (color, moisture, free acidity, pH, electrical conductivity, hydroxymethylfurfural (HMF) content, DPPH radical scavenging activity) were chosen as methods for honey characterization. The results of the melissopalynological analysis showed that all honey samples had a high percentage of Helianthus annuus pollen grains and these results help to classify the samples as monofloral honey. The values determined for electrical conductivity (314.82 - 440.55 µS/cm), color (32.87 - 47.52 mm Pfund), pH (3,65 - 4.34) confirmed the purity of the samples. In the case of moisture content, all the sunflower honey samples complied with the limit imposed by Codex Alimentarius (20%). The HMF content ranged between 2.66 and10.96 mg HMF/kg,which showed that the samples were fresh and had a HMF value below the limit of 40 mg HMF/kg. The DPPH radical scavenging activity ranged between 60.24 and 76.95 %. Gluconic acid was the organic acid predominant in all honey samples. These results led to an accurate classification of the analyzed honey according to its botanical origin, namely sunflower honey.*

Keywords: *honey, organic acids, physico-chemical properties*

1. Introduction

Honey has got well-known functional properties that determine its role in preventing disease, apart from being recognized as a natural product with multiple nutritional values. Its properties and also its extraordinary medicinal values can be guaranteed only if the product is authentic [1].

In Romania, the arable land used for agriculture represents over 60% of the total land of the country. Among crops, oilseeds (mainly *Brassica napus* and *Helianthus annuus*) account for about 10%. Other crops of importance for the country production include wheat, soybean, sugar beet, vegetables and vineyards. Flora and agricultural production determines which the most common types of Romanian honey are; these include acacia, sunflower, lime, raspberry, mint, chestnut, heather, honeydew and polyfloral honey. Sunflower honey is produced in large quantities in Romania because sunflower crops have been present in this region for a long time. Sunflower honey is an assortment of monofloral honey from Romania and it is widely consumed [2]. Honey is well known to consumers as a sweetener, but it is also a high quality food that is rich in beneficial substances (sugars, phenolic compounds, proteins, carotenoids, enzymes, vitamins, minerals, organic and free amino acids) essential for ensuring balanced biological processes.Therefore, the functional potential of honey has recently been throughly investigated [3]. Some studies could easily demonstrate the health benefits of honey of different botanical origin [4],[5], as well as the correlation between its benefitial effects on

human health and the levels of bioactive compounds, particularly polyphenols and phenolic compounds, contained by honey. Natural honey is a sweetener that presents high dietary value and an exclusive flavor character and therefore, it is more expensive than other sweeteners. This makes honey a frequent target of adulteration with the purpose of gaining increased economic profit. When compared to the adulteration of synthetic sweeteners, the most common fradulent practices in honey are relatively easy to achieve, but at the same time they can be difficult to detect. Considering that honey is the third most adulterated food product, authenticity of honey is of global concern for both commercial producers and consumers [6].

Honey is increasingly valuable for our diet due to its medicinal benefits, as it also has a high content of natural antioxidants. Honey also contains a small proportion of organic acids (0.5%), which can be used as indicators of deterioration, freshness, purity and authenticity. Organic acids are the result of aerobic and anaerobic fermentation and they are responsible for the special flavors of honey [7].

The aim of this study was to analyze the organic acids content and the physico-chemical parameters (melissopalynological analysis, moisture, pH, color, free acidity, electrical conductivity, hydroxymethylfurfural content, and antioxidant activity) of Romanian sunflower honey.

2. Materials and methods

2.1. Samples

Six samples of sunflower honey from 2018 from Romania were liquefied at 50 °C and homogenized to carry out the analysis.

2.2. Melissopalynological analysis

Pollen grains from sunflower honey samples were counted from a sediment spread on a microscopic slide using the light microscope (Motic ×40) [8], [9]. This sediment was obtained from 10 g of honey dissolved in 40 ml of distilled water. The resulting mixture was centrifuged at 4500 rpm for 15 min. The supernatant was removed, the residue obtained was redissolved and centrifuged for another 15 minutes. [8], [9].

2.3. Physico-chemical analysis

The physico-chemical analyses were performed for sunflower honey samples according to the analytical methods harmonized by the International Honey Commission [10]. The physico-chemical characteristics analyzed for honey samples were the following:

2.3.1.Moisture content

The Abbe refractometer Leica Mark II Plus (Leica, USA) was used to determine the moisture content for liquefied sunflower honey samples. Liquefaction took place at a temperature of 50 ºC. The Chataway table [11] was used to calculate water content (%).

2.3.2. Electrical conductivity

Electrical conductivity was measured using a portable conductometer HQ14d, HACH (USA).

2.3.3. pH

pH meter METTLER TOLEDO FiveGo, Mettler Toledo, (SUA) was used to analyze the pH of samples.

2.3.4. Free acidity

Free acidity was measured using TITROLINE easy, Schott Instruments (Germany).

2.3.5. Color

Color was analyzed using the photometer Pfund, Hanna Instrumets HI 96785 and the portable chromameter CR-400, Konica Minolta, (Japan).

2.3.6. Hydroxymethylfurfural (HMF)

HMF content was determined with a spectrophotometer UV-VIS-NIR SCHIMADZU UV-3600, (Schimadzu Corporation, Japan) using the White method[12].

2.3.7. DPPH assay

The determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was made, as follow: 1 g of honey was dissolved in 5 mL of methanol 40% (v/v, with acidified water) and stirred for 15 min with a magnetic stirrer [13]. Then, 35 µL of honey solution were mixed with 250 µL of DPPH. The absorbance was measured using a QE65000 spectrometer (Ocean Optics, SUA) at 515 nm. The results were obtained using the formula in Eq. 1:

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

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

2.4.Determination of organic acids

0.5 g of sunflower honey was mixed with 2.5 mL of 4% metaphosphoric acid (w/v) and then were vortexed. They were determined according to the method proposed by Özcelik et al. [14].

3. Results and discussion

Pollen analysis

Pollen analysis is the traditional method proposed by the International Bee Botanical Commission (ICBB) in 1970 [11]. Using the microscopic analysis we can determine the plants visited by bees during honey production [15].

The *Helianthus annuus* pollen was predominant in all the samples, accounting for more than 45% pollen grains (50-92 % pollen grains). Oroian & Ropciuc [16] reported in a different study made on sunflower honey that their samples had around 60% of pollen belonging to *Helianthus annuus.*

Moisture content

Processing and storage conditions influence the moisture content of honey along with the relative moisture in the region of honey origin [17]. The honey samples that have a high moisture content may be unstable during storage. Thus, the moisture content is used as an indicator of honey quality and stability, and Codex Alimentarius has set an allowable limit of 20% [18, 19].

The moisture content of the analyzed samples ranged from 16.23 % to 20.39%. These results were in accordance with the values (17.3%) reported by Oroian et al.[20] when analyzing sunflower honey.

In Serbian sunflower honey the moisture content varied between 15.5 and 20.63% [21]. Do Nascimento et al.[22] reported for forty-nine monofloral honey samples a variation of moisture content from 16.4% to 19.4%. (3)

pH

Normally the pH of honey is between 3.5 and 5.5 due to the presence of organic acids that give both the aroma of honey and protection against microbial damage [6]. pH values contribute to the identification of honey botanical origin [23].

pH of the sunflower honey samples ranged from 3.65 to 4.34. Kukurová et al. [24] reported for sunflower honey a pH of 3.7 and Terrab et al.[25] reported pH values between 3.56 and 4.79 in a study of Spanish thyme honey. Manzanares et al. [26] reported values between 3.70 and 5.15 for one hundred eighty and two samples of honey samples from Tenerife.

Table 1

Physico-chemical parameters of sunflower honey

	Sunflower Honey						
Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	F -ratio
L*	33.59(0.47)e	49.53(0.70)a	28.5(0.41)f	42.47(0.60)c	40.14(0.57)d	42.78(0.61)b	337.24***
h^*_{ab}	82.37(1.17)cd	88.07(1.25)a	78.37(1.11)f	80.21(1.14)de	83.97(1.20)b	83.08(1.18)cd	15.94**
C^*_{ab}	23.08(0.33)d	29.9(0.42)b	18.32(0.26)f	21.51(0.30)e	25.75(0.36)c	30.38(0.43)a	353.38***
Color (mm Pfund)	33.66(0.48)e	39.10(0.55)c	35.64(0.50)d	47.52(0.67)a	32.87(0.18)e	43.56(0.62)b	1051.47***
pH	4.04(0.05)b	3.65(0.04)e	3.83(0.05)c	4.12(0.05)b	3.78(0.05)cd	4.34(0.06)a	40.11**
Free acidity (meq/kg)	18.31(0.26)e	47.32(0.37)a	33.46(0.48)c	30.49(0.43)d	45.64(0.65)b	15.94(0.22)f	1463.58***
Electrical conductivity (mS/cm)	314.82(4.49)d	322.74(4.61)d	341.05(4.87)c	320.76(4.58)d	378.18(5.40)b	440.55(6.29)a	183.55***
Moisture (%)	16.95(0.24)c	20.31(0.28)a	20.39(0.28)a	17.46(0.24)bc	17.78(0.25)b	16.23(0.23)d	91.13***
HMF (mg/kg)	10.96(0.15)a	2.66(0.03)f	3.40(0.04)e	4.44(0.06)d	7.85(0.11)c	9.48(0.13)b	2259.65***
DPPH	69.99(0.99)b	60.02(0.85)d	76.59(1.09)a	76.95(1.09)a	66.67(0.95)c	60.24(0.86)d	117.26***

Table 2

Organic acids of sunflower honey

	Sunflower Honey						
Organic acids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	F -ratio
Gluconic acid	3.99(0.05)d	5.62(0.08)a	4.52(0.06)c	4.79(0.06)b	4.01(0.05)d	4.38(0.06)c	170.93***
Formic acid	0.098(0.001)f	0.35(0.005)c	0.33(0.004)d	0.75(0.010)a	0.70(0.01)b	0.30(0.004)e	2680.3***
Acetic acid	0.85(0.01)a	0.51(0.007)b	0.45(0.006)c	0.15(0.002)e	0.14(0.002)e	0.34(0.004)d	3030.38***
Propionic acid	0.64(0.009)c	0.55(0.007)d	0.43(0.006)f	0.79(0.011)b	0.50(0.007)e	0.91(0.01)a	730.95***
Lactic acid	0.06(0.0009)c	0.08(0.001)b	0.04(0.0006)d	0e	0e	0.18(0.002)a	5828.02***
Butyric acid	0.10(0.001)c	0.36(0.005)a	0.13(0.001)b	0.35(0.005)a	0d	0d	5465.39***
Succinic acid	0.05(0.0008)e	0.15(0.002)b	0.03(0.0004)f	0.08(0.001)c	0.06(0.0009)d	0.15(0.002)a	2453.02***

ns – not significant ($p > 0.05$), *** – $p < 0.001$

[a-c] – different letters in the same row indicate significant differences between samples ($p < 0.05$)

Free acidity

The analysis of free acidity is preferred to determine the freshness of honey. Along with the deterioration of honey, the value of free acidity increases as a result of the fermentation of sugars into organic acids.

Thus, free acidity depends directly on the organic acids present in honey, but also on the harvesting season as well as on the geographical origin [17]. 50 milliequivalents of acid per 1,000 grams is the maximum limit allowed by legislation for free acidity [27].The free acidity of sunflower honey analyzed in this study ranged from 15.94 to47.32 meq/kg. Matović et al. [28] reported for Serbian honey a value of 23.64% and Geană et al. [29] reported for Romanian sunflower honey a free acidity value of 35.1 %.

HMF content

Alongside free acidity, the analysis of HMF content is a parameter used to indicate the degree of freshness of honey. 40 mg/kg is the limit imposed by the legislation for HMF. The samples that exceed this value have been subjected to strong or prolonged heat treatment or they have been improperly stored [30], [18], [27].

All the honey samples analyzed in this study had a low HMF content. Matović et al.[28] reported a value of 2.69 mg/kg for Serbian honey samples and Yardibi & Gumus [31] reported an average HMF content of 7.02 mg/kg in the honey samples analyzed in their study.

Color

The first quality attribute evaluated by consumers is the color of honey that must fall within a range expected and accepted by consumers [32],[33]. Color is often used to distinguish variations that may be due to geographical origin, botany or production particularities [34]. By visual color analysis of honey, the botanical origin of this product, as well as the degree of heat treatment and the presence of flaws such as fermented products can be assessed [35].

The color of sunflower honey ranged from white (32.87 mm Pfund) to extra light amber (47.52 mm Pfund).

Kadar et al.[36] reported for sunflower honey a value of 58.11 mm (light amber color).Juan-Borrás et al.[37] reported for Romanian sunflower honey a value of 51 mm and the value of 66.7 mm was reported for sunflower honey from Spain. All honey samples showed similar lightness values (28.5-49.53).

Electrical conductivity

Electrical conductivity is a parameter that provides information on the botanical origin of honey. Electrical conductivity is positively correlated with the ash content and acidity due to the presence of ions, acids and organic proteins [38]. Minerals are brought in honey primarily with pollen, so the electrical conductivity correlates with the pollen content of monofloral honey [39], which determines the botanical origin [40]. As the values in Table 1 show, the sunflower honey samples analyzed had an electrical conductivity less than 500 µS/cm, therefore these samples were of pure floral honey. Devillers et al. [41] reported for sunflower honey a mean value of 306.2 µS/cm. In our study the values ranged between 314.82 to 440.55 µS/cm. Kádár et al. [36] reported for sunflower a value of 420 µS/cm and for acacia honey 220 µS/cm. Matović et al.[28] reported for Serbian honey a value of 220 µS/cm.

DPPH assay

The DPPH test was used because high DPPH purification activity provides the sample with a superior antioxidant activity [42]. In this study the sunflower honey had a DPPH value between 60.24 and 76.95 %.Đogo Mračevića et al.[43] reported for sunflower honey values between 33.18% and 40.18 %.Romanian sunflower honey had a higher antioxidant activity than those in Serbia investigated by Đogo Mračevića.

Organic acids content

Organic acids are very important because they contribute to the preservation and assessment of the sensory properties of honey [17]. Gluconic acid was the predominant acid in all the sunflower honey samples analyzed in this study. The maximum gluconic acid content was determined in sample 2 (5.62 g/kg) and the lowest value in sample 1 (3.99 g/kg). Sahin et al. [44] identified gluconic acid as the predominant acid in all the honey samples that they studied, and the maximum gluconic acid content was reported in chestnut honey (8.90 g/kg). Formic, acetic propionic and succinic acids were also present in all sunflower honey samples in this study, but in smaller quantities. Sahin et al. [44] reported the presence of formic acid in almost all the samples. In their study, Suarez-Luque et al.[45], reported a low concentration of citric, malic, succinic and fumaric in eucalyptus honey samples and a high concentration in chestnut honey.

4. Conclusion

The physico-chemical characterization, pollen analysis and organic acids content of sunflower honey samples collected from Romania were performed to examine the quality of honey samples. These analyses were used to determine the botanical origin of honey. The six honey samples met the quality criteria examined (electrical conductivity, color, moisture, pH, free acidity, and HMF content), and together with the pollen analysis and organic acids content indicated that the honey samples analyzed were monofloral sunflower samples.

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