CHARACTERIZATION OF SERBIAN SUNFLOWER HONEYS BY THEIR PHYSICOCHEMICAL CHARACTERISTICS

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Abstract: Five physicochemical parameters (water content, electrical conductivity, total acidity (pH), ash mass fraction and concentration of free acids) were analyzed in 15 sunflower honeys collected from several localities in Vojvodina, Serbia. The mean values of analyzed honeys were: water content 16.87%; concentration of free acids 27.43 mEq/kg; electrical conductivity 0.34 mS/cm; pH 3.64 and ash mass fraction 0.13%. The selected physicochemical characteristics of all honey samples from Serbia analyzed in this research can be considered to be within the parameters expected for sunflower honey in general. The values for ash mass fraction, electrical conductivity and concentration of free acids in all sunflower honey samples showed similar trends. High correlation was established between electrical conductivity and ash mass fraction. Statistically significant difference (p≤0.05) was established only for average values of free acids concentration between honey samples originating from the localities Kanjiža and Čelarevo. All of the analyzed honey samples were found to meet national and European legislation for investigated parameters

Key words: sunflower honey, electrical conductivity, acidity, ash

INTRODUCTION

Honey is natural food that contains about 200 substances. Besides its main components, glucose and fructose, honey contains water and other substances such as proteins, organic acids, vitamins, minerals, pigments, phenolic compounds, volatile compounds, and solid particles derived from honey harvesting (Bogdanov, Jurendić, Sieber & Gallmann, 2008; Da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). The composition, color, aroma and flavor of honey depend mainly of the flower source, climatic conditions, geographical locations, honeybee species, weather conditions, processing, manipulation and storage time (Escuredo, Dobre, Fernández-González & Seijo, 2014; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). The physicochemical properties of honey with same floral origin can vary as consequence of different climatic conditions or geographical origin (Juan-Borrás, Domenech, Hellebrandova, & Esriche, 2014).

Honey physicochemical quality criteria are well specified by the European legislation (European Commission, 2002) and the regulation concerning the quality of honey in the Republic of Serbia (Pravilnik, 2015). The major criteria for honey quality and authenticity, in both of those standards are sugar content (sucrose and sum of fructose and glucose),
moisture content, water-insoluble matter content, electrical conductivity, free acidity, diastase activity and hydroxymethylfurfural (HMF) content. The classical approach to the determination of honey botanical origin includes sensory, melissopalynological and physicochemical analysis (European Commission, 2002). This approach is time-consuming and requires personnel specialized in carrying out sensory and melissopalynological analysis (Lazarčević, Andrić, Trifković, Tešić & Milojković-Opšenica, 2012). Many studies have been carried out to find analytical markers of the botanical and geographical origin of honey. In these studies, physicochemical parameters (Devillers, Morlot, Pham-Delégue & Doré, 2004; Acquarone, Buera & Elizalde, 2007; Kirs, Pall, Martverk, & Laos, 2012; Juan-Borrás et al., 2014; Karabagias et al., 2014; El Sroieh, Masry & Shehata, 2002; Da Silva et al., 2016), carbohydrate profiles (Kaškonienė, Venskutonis & Ėckstirtytė, 2010; Esccero et al., 2014; Živkov Balolaš, Popov, Prodanoav Radulovčić, Stojanov & Jakšić, 2020), mineral content (Lazarčević, 2016; Mračević, Krštić, Ločić & Ražić, 2020), amino acid composition (Sakač et al., 2019), phenolic content (Sari & Ayyildiz, 2012; Alqarni, Owayss, & Mahmoud, 2012; Cimpoiu, Hosu, Miclaus, & Puscas, 2013), volatile compounds (Juan-Borrás et al., 2014) have been suggested as criteria for the characterization of honeys.

The electrical conductivity (EC), total acidity (pH), and ash mass fraction are related to the concentration of mineral salts, organic acids and proteins (Acquarone et al., 2007; Lazarčević, Jovetić, & Tešić, 2017). The EC is very often used in routine quality control of honey. This parameter is defined as that of a 20% weight in volume solution in water at 20°C, where the 20% refers to honey dry matter (International Honey Commission Methods, 2009). It is used to distinguish between floral and honeydew honeys according to the standard. In accordance with the regulation concerning the quality of honey in the Republic of Serbia (Pravilnik, 2015), the minimum EC in honeydew placed on the market is set at 0.8 mS/cm. For other types of honey, the maximum permitted value of EC is 0.8 mS/cm. This parameter is also important for the authentication of unifloral honeys (Kaškonienė et al., 2010). Other factors such as floral source, amount of organic acids and proteins, and storage time can also influence the electric conductivity of honey (Karabagias et al., 2014). High electric conductivity values do not necessarily correspond to higher amounts of ash in the honey (Escuerdo et al., 2014). The EC value depends on the ash and acid content in honey: the higher their content, the higher the resulting EC (International Honey Commission Methods, 2009). The ashes of honey give an indication of environmental pollution and hence also an indication of geographical origin. The reference value for the ash mass fraction is not prescribed by the regulations.

The acidity of honey causes the presence of organic acids (tartaric, citric, oxalic, acetic, etc.), nectar or secretions of bees (Yadata, 2014). The acidity of honey can be determined by titration with sodium hydroxide (free acidity) or directly measuring the pH value (pH value). The natural acidity of honey can be increased by the storage and ripening of honey, as well as during the fermentation of honey. Honey that is adulterated with sugar syrup has very low acidity (<1), while honey that is adulterated with invert sugar has a pronounced high acidity (Yadata, 2014). The acidity value related to the balance of organic acids naturally present in honey varies according to the floral source and the bee species (Sousa et al., 2016). The maximum value of free acidity in all types of honey (except in baker's honey) is 50 mEq/kg (Pravilnik, 2015). The reference value for the total acidity is not prescribed by the national regulations.

Sunflower honey is bright yellow, fragrant-smelling, dry, with an aroma of pollen, slightly herbaceous. This is a traditional honey with exceptional healing properties and nutritional value. It is discovered that sunflower pollen consistently reduced a protozoan pathogen (*Crithidia bombi*) infection in bumble bees and also reduced a protozoan pathogen (*Nosema ceranae*) of the honey bee (*Apis mellifera*) indicating the potential for broad antiparasitic effects (Giacomini et al., 2018). Sunflower honey contains small percentage of sucrose and therefore gets quickly crystallized (Šrimač & Plairuk, 2009; Sari, & Ayyildiz, 2012). Sunflower is cultivated only in the southern regions, where there is a lot of sun and where the climate is favorable for growing this plant. In Serbia, honey production is well developed thanks to a suitable climate and geographic location. The beekeeping, which has been sig-
nificantly developed and maintained in Serbia since the 10th century, is an important agricultural activity (Lazarević, 2016).

The purpose of this study was to determine water content, electrical conductivity, ash and acidity of sunflower honey samples collected from beekeepers in different regions of Vojvodina (northern Serbia), in order to obtain information about the honey quality and safety. In our research, which is part of a study of the physicochemical and sensory characteristics of Serbian unifloral and mixed honey, the behavior of honey was specifically examined with respect to the electrical conductivity and free acidity.

**MATERIALS AND METHODS**

**Honey samples**

A total of 15 samples of sunflower honey harvested in 2019 were collected from beekeepers in different regions of the Autonomous Province of Vojvodina, Serbia. The sampling included locations in municipalities Kikinda (3 samples), Čelarevo (7 samples) and Kanjiža (5 samples). All collected samples were in their original packaging (jars) and transferred to the laboratory of Scientific Veterinary Institute “Novi Sad” for examination. Manufacturers used field observations for botanical origin determination. Our research included only the samples with confirmed botanical origin stated on the manufacturing specification label. All selected samples were produced by *Apis mellifica*. Honey analyses were carried out immediately after sampling. The honey analysis procedures were performed in two replicates.

**Physicochemical analysis**

**Water content**

Water content was determined by refractometry, measuring the refractive index (RI) according to the harmonized methods of the International Honey Commission (International Honey Commission Methods, 2009) using a standard model Abbe type refractometer at 20°C. Water content (%) was then obtained from the Chataway table (International Honey Commission Methods, 2009).

**Electrical conductivity**

Electrical conductivity was measured at 20°C in solutions of honey samples (20.0 g dry matter of honey in volume solution in 100 ml distilled water) using a conductometer Crison (Type Basic 30). The method of measuring is prescribed by International Honey Commission Methods (2009).

**Free acidity and pH**

The acidity of honey was determined by volumetric method (International Honey Commission Methods, 2009). Ten grams of honey were dissolved in 75 ml of distilled water, the pH is measured and solution was titrated with 0.1 M NaOH to pH 8.30. Acidity is expressed in milliequivalents/kg honey (mEq/kg).

**Ash mass fraction**

The ash content of honey means the residue which is obtained by a defined procedure and expressed as a percentage by weight (International Honey Commission Methods, 2009). The honey is ashed at 600°C and the residue is weighed.

**Statistical analysis**

Statistically significant differences between the individual parameters at different locations were determined using one-way ANOVA (PAST, Version 2.12, Oslo, Norway). Statistical linear regression analysis was performed by the PAST software package, version 2.12, Oslo, Norway. Data were presented as mean, standard deviation, minimum and maximum value.

**RESULTS AND DISCUSSION**

Average values of water content, electrical conductivity, acidity, pH and ash mass fraction of honey obtained in this study are shown in Table 1. The obtained values were compared with the values that are prescribed by the Regulation on the quality of honey in the Republic of Serbia (Pravilnik, 2015). Also, the results were compared with the results from other authors from both Serbia and other countries. ANOVA one way statistical analysis revealed statistically significant difference between the localities Kanjiža and Čelarevo at p≤0.05 only for free acidity of honey.

The water content values in all investigated honey samples ranged between 14.60 – 18.60% (average 16.87 ± 1.23%). The water content in all investigated honey samples was below the maximum permitted level (20%) set by regulations for honeys (European Commission, 2002; Pravilnik, 2015). The honey moisture in
depends on the production season, floral source, abundance of nectar flow, soil, ventilation of the beehive, colony strength, and meteorological conditions in the area of honey production, primarily air humidity (Kirs, Pall, Martverk, & Laos, 2011; Escuerdo et al., 2014; Sousa et al., 2016; Lazarević et al., 2017). An important factor that could affect the water content is honey maturation and harvest time. According to most of national good beekeeping practice recommendations, beekeeper should harvest honey when at least 2/3 wax combs in frames are covered with wax lids (Živkov Baloš, Jakšić, Popov, Mihaljev & Ljubojević Pelić, 2019).

Values of electrical conductivity in the investigated honey samples were between 0.22 and 0.54 mS/cm (average 0.34 ± 0.09 mS/cm), which is also below maximum permissible value (0.8 mS/cm). The data indicate that the lowest average conductivity was in honey samples from the locality Kanjiža, while the highest average conductivity was measured in samples from locality Čelarevo. Similar values for electrical conductivity in sunflower honey were reported by other authors from Serbia, Greece, France, Argentina, Spain, Romania, and Czech Republic (Thrasyvoulou & Manikis, 1995; Devillers et al., 2004; Acquarone et al., 2007; Juan-Borrás et al., 2014; Lazarević et al., 2012; Mračević et al., 2020). Ash mass fraction is an indicator of the mineral content of honey. This parameter mainly depends on soil and climate characteristics (Acquarone et al., 2007).

| Sample | Water (%) | Electrical conductivity (mS/cm) | Free acidity (mEq/kg) | pH | Ash mass fraction (%) |
|--------|------------|---------------------------------|-----------------------|----|-----------------------|
| Ka 1   | 18.60      | 0.32                            | 24.40                 | 3.57 | 0.08                  |
| Ka 2   | 16.40      | 0.28                            | 20.40                 | 3.69 | 0.05                  |
| Ka 3   | 18.40      | 0.30                            | 20.80                 | 3.52 | 0.05                  |
| Ka 4   | 15.20      | 0.28                            | 23.80                 | 3.55 | 0.12                  |
| Ka 5   | 14.60      | 0.30                            | 22.40                 | 3.99 | 0.14                  |
| C 1    | 17.40      | 0.36                            | 27.20                 | 3.64 | 0.11                  |
| C 2    | 16.40      | 0.54                            | 30.00                 | 3.63 | 0.21                  |
| C 3    | 16.40      | 0.42                            | 36.40                 | 3.61 | 0.13                  |
| C 4    | 16.00      | 0.22                            | 26.80                 | 3.46 | 0.09                  |
| C 5    | 15.40      | 0.26                            | 24.50                 | 3.59 | 0.09                  |
| C 6    | 17.80      | 0.44                            | 34.40                 | 3.84 | 0.25                  |
| C 7    | 18.20      | 0.46                            | 35.60                 | 3.62 | 0.30                  |
| Ki 1   | 17.80      | 0.30                            | 26.40                 | 3.63 | 0.09                  |
| Ki 2   | 17.60      | 0.32                            | 32.20                 | 3.70 | 0.09                  |
| Ki 3   | 16.80      | 0.30                            | 26.20                 | 3.68 | 0.10                  |

Table 1.
Results of water content, electrical conductivity, acidity, pH and ash mass fraction in 15 sunflower honey samples from locations in municipalities Kanjiža, Čelarevo and Kikinda

| Sample | Water (%) | Electrical conductivity (mS/cm) | Free acidity (mEq/kg) | pH | Ash mass fraction (%) |
|--------|------------|---------------------------------|-----------------------|----|-----------------------|
| Ka 1   | 18.60      | 0.32                            | 24.40                 | 3.57 | 0.08                  |
| Ka 2   | 16.40      | 0.28                            | 20.40                 | 3.69 | 0.05                  |
| Ka 3   | 18.40      | 0.30                            | 20.80                 | 3.52 | 0.05                  |
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| Ka 5   | 14.60      | 0.30                            | 22.40                 | 3.99 | 0.14                  |
| C 1    | 17.40      | 0.36                            | 27.20                 | 3.64 | 0.11                  |
| C 2    | 16.40      | 0.54                            | 30.00                 | 3.63 | 0.21                  |
| C 3    | 16.40      | 0.42                            | 36.40                 | 3.61 | 0.13                  |
| C 4    | 16.00      | 0.22                            | 26.80                 | 3.46 | 0.09                  |
| C 5    | 15.40      | 0.26                            | 24.50                 | 3.59 | 0.09                  |
| C 6    | 17.80      | 0.44                            | 34.40                 | 3.84 | 0.25                  |
| C 7    | 18.20      | 0.46                            | 35.60                 | 3.62 | 0.30                  |
| Ki 1   | 17.80      | 0.30                            | 26.40                 | 3.63 | 0.09                  |
| Ki 2   | 17.60      | 0.32                            | 32.20                 | 3.70 | 0.09                  |
| Ki 3   | 16.80      | 0.30                            | 26.20                 | 3.68 | 0.10                  |

Table 2.
Correlation between examined parameters in sunflower honey from Serbia

| Linear regression between:          | Regression Equation | R² |
|------------------------------------|---------------------|----|
| electrical conductivity            | ash mass fraction   | y = 0.945x+0.220 | 0.611 |
| electrical conductivity            | free acidity        | y = 41.12x+13.45  | 0.476 |
| free acidity                       | ash mass fraction   | y = 50.81x+20.99  | 0.497 |
Generally, the ash content is ≤ 0.6% (w/w) (Pita-Calvo & Vásquez, 2017). Obtained values for ash mass fraction in sunflower honeys varied from 0.05 to 0.30%. Average ash mass fraction value was 0.13 ± 0.07%. High linear correlation between EC and ash mass fraction was found by many authors (Accorti, Piazza & Persano-Oddo, 1987; Thrasyvoulou, & Manikis, 1995; Kropf, Jamnik, Bertoncelj & Golob, 2008; El Sohaimy, Masry, & Shehata, 2015; Lewoyehu & Amare, 2019).

Free acidity in all samples tested was below 50 mEq/kg, indicating the absence of undesirable fermentation. The acidity value in the investigated sunflower honey samples ranged from 20.40 mEq/kg to 36.40 mEq/kg (average 27.43 ± 5.19 mEq/kg), which is in line with the results reported by Devillers et al. (2004), Lazarević et al. (2012), Prica et al. (2014) and Mračević et al. (2020). Free acidity and pH increases with storage time, as well as during fermentation, because honey sugars and alcohols transform into acids by honey yeasts and bacteria (Cavia, Fernández-Muñoz, Alonso-Tore, Huidobro, & Sancho, 2007; Alqarni et al., 2012). Honey pH depends on the content of organic acids and mineral elements and influences microorganisms development, enzymatic activity and texture, among other properties (Cavia et al., 2007; Pita-Calvo & Vásquez, 2017). As visible from Table 1, the pH values in analyzed honey samples ranged from 3.46 to 3.99 (average 3.64 ± 0.13). This range is consistent with the results for sunflower honey obtained by other authors (Thrasyvoulou & Manikis, 1995; Sari & Ayyildiz, 2012; Lazarević et al., 2012; Cimpoiu et al., 2013; Mračević et al., 2020). The pH values of honey usually range between 3.5 and 5.5 (Pita-Calvo, & Vásquez, 2017).

The ash mass fraction, electrical conductivity and free acidity values of all sunflower honey samples showed similar trends (Figure 1). Since investigated properties are a function of the concentration of the ions present in the honey, their correlation was examined (Table 2). It was found that there is a correlation between electrical conductivity and ash mass, acidity and ash mass, acidity and electrical conductivity. Between other examined parameters, the correlation coefficients were insignificant. Correlation was established between electrical conductivity and ash mass fraction ($R^2=0.611$). Correlation between free acidity and electrical conductivity calculated for sunflower honey samples was $R^2=0.476$. In our earlier study that included 55 honey samples (16 samples of acacia honey, 18 samples of meadow honey, 9 samples of multifloral honey, 6 samples of linden honey, and 6 samples of honeydew honey), significant linear correlation between free acidity and electrical con-
ductivity was established only for meadow and multifloral honey samples ($R^2 = 0.8210$ and $R^2 = 0.7688$). A similar correlation coefficient to that obtained for sunflower honey was found for honeydew honey ($R^2=0.4996$) (Živkov Baloš et al., 2018).

**CONCLUSIONS**

The water content, electrical conductivity, total acidity, ash mass fraction and concentration of free acids of sunflower honey collected from beekeepers in different regions of the Autonomous Province of Vojvodina, Serbia was determined. Our results demonstrated that Serbian sunflower honey is characterized by good quality since the examined parameters closely related to the quality of honey, showed values in agreement with legal limits established by the national and European legislation. The hypothesis about potential existence of a correlation between the parameters tested was confirmed for electrical conductivity/ash mass, acidity/ash mass fraction, as well as for acidity/electrical conductivity. Likewise, we found that these parameters show similar trends. By observing the geographical characteristics of Serbian sunflower honey, we found statistically significant differences only between average values of free acidity of honey originating from localities of Kanjiža and Čelarevo.

Therefore, further research on physicochemical properties of sunflower honey is required to confirm the quality and authenticity of this product. The importance of such research lies in its contribution to better understanding the benefits of this type of honey.

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Sažetak: U ovom radu analizirano je pet fizičko-hemijskih parametara (sadržaj vode, električna provodljivost, ukupna kiselost, maseni udeo pepela i koncentracija slobodnih kiselina) u 15 uzoraka sunčokretovog meda, prikupljenih sa nekoliko lokaliteta Vojvodine. Srednje vrednosti ispitivanih parametara bile su: voda 16,87%; koncentracija slobodnih kiselina 27,43 mEq/kg; električna provodljivost 0,34 mS/cm; maseni udeo pepela 0,13% i pH 3,64. Fizičko-hemijske karakteristike svih ispitanih uzoraka meda mogu se smatrati u okvirima vrednosti koji se očekuju za sunčokretov med. Maseni udeo pepela, električna provodljivost i koncentracija slobodnih kiselina u svim uzorcima meda sunčokreta pokazali su slične trendove. Utvrđena je visoka korelacija između električne provodljivosti i masenog udela pepela. Samo prosečne vrednosti koncentracije slobodnih kiselina meda, su se statistički značajno razlikovale i to između lokaliteta Kanjiža i Čelarevo (r = 0,017). Na osnovu dobijenih vrednosti za ispitivane parametre, utvrđeno je da svi uzorci sunčokretovog meda ispunjavaju uslove nacionalnog i evropskog zakonodavstva.dehidrirane pečurke mogu se smatrati novim funkcionalnim (polu)proizvodima, uzimajući u obzir njihov poboljšan nutritivni profil.

Ključne reči: sunčokretov med, električna provodljivost, kiselost, pepeo

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