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Comparative analysis of antibacterial and antioxidant activity of three different types of honey

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A B S T R A C T

Honey is a natural product which has high nutritional value. Also, it has health benefits, which is the reason for its been used for the prevention of diseases caused by oxidative stress for centuries in many countries. Honey has antibacterial and antioxidant properties, which are the result of the presence of enzymes and chemical and phytochemical components, where phenolic acids and flavonoids play a significant role. The aim of this study was to examine the physico-chemical parameters, antioxidant properties and antimicrobial activity of forest-meadow, acacia and sunflower honey from the Gruža and Požega areas (Republic of Serbia). The tested physico-chemical parameters were found to be within the values prescribed by the Legislation and the Codex Alimentarius Standard. The presence of HMF was not detected in the sample of forest-meadow honey, while the content in other samples (1.09 and 1.79 mg HMF kg^{-1}) was significantly below the value laid down by regulations (40 mg HMF kg^{-1}). Forest-meadow honey had the highest antioxidant activity (35.2%, 26.32% – ABTS and DPPH method, respectively) and the highest content of total phenolics (90.96±5.18 g GAE 100 g^{-1}), while acacia honey exhibited the lowest values for antioxidant activity (11.76% – ABTS; 7.28% – DPPH) and total phenolics (15.75±0,66 g GAE 100 g^{-1}). Forest-meadow honey showed the best antibacterial activity, followed by sunflower and acacia honey.

Keywords: honey, HMF, phenolics, antioxidant properties and antibacterial activity.

И З В О Д

Мед има антибактеријска и антиоксидативна својства која су последица присуства ензима, хемијских и фитохемијских компоненти, при чему фенолне киселине и флавоноиди имају најважнију улогу. Циљ истраживања био је испитивање неких физичко-хемијских параметара, антиоксидативних својстава и антимикробне активности три узорка меда (шумско-ливадски, багремов и сунцокретов) из околине Груже и Пожеге (Република Србија). Утврђено је да су испитивани физичко-хемијски параметри у оквиру вредности прописаних Правилником и Codex Alimentarius стандардом. У узорку шумско-ливадског меда није детектовано присуство HMF, док је садржај у осталим узорцима (1,09 и 1,79 mg HMFkg^{-1}) био значајно испод вредности прописане регулативама (40 mg HMFkg^{-1}). Шумско-ливадски мед је показао највећу антиоксидативну активност (35,2% – ABTS; 26,32% – DPPH) и највећи садржај укупних фенола (90,96±5,18 g GAE 100 g^{-1}), док је багремов мед показао најнижу антиоксидативну активност (11,76% – ABTS; 7,28% – DPPH) и најмањи садржај укупних фенола (15,75±0,66 g GAE 100 g^{-1}). Најбољу антибактеријску активност показао је шумско-ливадски, затим сунцокретов, а најслабију багремов мед.

Кључне речи: мед, HMF, феноли, антиоксидативна и антибактеријска својства.

1. Introduction

Honey a complex food product. It has been used as medicine and as a food source for centuries. According to Codex Alimentarius and EU Directive 110/2001 bees (*Apis mellifera*) collect nectar or plant secretions and turn them into a natural substance called honey (Pauliuc et al., 2020). Honey gets its sweetness from the monosaccharides glucose and fructose. The specific taste of honey comes from the high concentration of carbohydrates, and the aroma comes from the type of nectar. The color of honey can vary from almost colorless to dark brown. The color depending on the type of honey (Brudzynski and Miotto, 2011; Rao, 2016).

Honey has significant nutritional value because it contains essential minerals, free amino acids and proteins, as well as vitamins, enzymes and phytochemicals (Alvarez-Suarez et al., 2010; Cianciosi et al., 2018). The chemical composition and quality of honey depends on the botanical and geographical origin, the types of plants, climatic and environmental conditions such as temperature, humidity, etc. It is known that honey has therapeutic properties thanks to the antioxidants present in honey such as various phenolic acids, flavonoids, flavonols, carotenoids, α-tocopherol, ascorbic acid, amino acids, enzymes, etc.

(Savatović et al., 2011; Noori et al., 2014). Various compounds present in honey cause antioxidant activity. Among them are compounds of phenolic nature, peptides, organic acids. Also, the products of Maillard reactions can have an antioxidant capacity.

Many studies have shown that honey has an antimicrobial effect on various pathogenic strains of bacteria and fungi. The main component in honey with antimicrobial properties is hydrogen peroxide. Also, the factors responsible for the antimicrobial activity of honey can be the high concentration of sugar, the low pH, glucose oxidase, catalase, 1,2-dicarbonil compound methylglyoxal, lysozyme (Chang et al., 2011). The antibacterial effect is related to their phenolic composition, i.e. phenolic compounds are thought to act on cell membranes or enzymes (Chang et al., 2011). Flavonoids are a group of pigmented plant products that significantly contribute to the antimicrobial activity of honey (Nassar et al., 2012). The antimicrobial activity of flavonoids is the result of inhibition of nucleic acids and damage to the cytoplasmic membrane and cell wall (Cushnie et al., 2011). The antimicrobial properties of honey are related to the type of honey and are the result of readily volatile substances – phytoncides. Honey has a bactericidal and bacteriostatic effect on most Gram-positive bacteria (Molan, 1992; Molan, 1997; Bogdanov, 1997). Various substances present in honey are responsible for its antimicrobial effect. The botanical origin affects the antimicrobial properties of honey. Darker types of honey are more inhibitory than lighter honey.

The aim of this study was to determine some physico-chemical, antioxidant and antibacterial properties of different types of honey: forest-meadow, acacia and sunflower honey.

2. Materials and methods

2.1. Honey samples

Honey samples (3 replicates per sample) were domestically produced, with the first two samples, forest-meadow honey and acacia honey, originating from the village of Pajsijević near Gruža (43°50'14.0"N, 20°43'34.6"E, 233 m a.s.l). The third sample, sunflower honey, originated from the village of Visibaba, the vicinity of Požega (43°50'3.01"N, 20°0'42.01"E, 360 m a.s.l), it was a migratory apiary, the geographical area of the place where the honey was collected was Padina (Kovačica), Banat region (43°18'46.01"N, 21°31'30"E). Forest-meadow honey was from 2020, while acacia and sunflower honey were from 2018, and both samples were crystallized (Table 1).

Table 1.
Types and origin of tested honey samples

Samples	Type	Origin
I	Forest-meadow honey	Gruža (central Serbia)
II	Acacia honey (crystallized)	Gruža (central Serbia)
III	Sunflower honey (crystallized)	Požega (western Serbia)

2.2. Determination of physico-chemical parameters

2.2.1. Determination of water and soluble dry matter content

The content of water and soluble dry matter content in honey was determined using a refractometer. A homogeneous sample was taken for analysis, and if crystallized, it was heated to a liquid state and then measured on a refractometer.

2.2.2. *Determination of hydroxymethylfurfural (HMF) content in honey*

The content of hydroxymethylfurfural was determined by the spectrophotometric method (White, 1979). The absorbances were then measured on a UV-VIS spectrophotometer (Cary Series 300, Agilent Technologies, USA) at wavelengths λ = 284 and 336 nm.

2.3. Determination of total phenolic content (TPC) and antioxidant capacity

The extraction was performed with water at room temperature (2 g of honey was dissolved in 10 mL of water). Such extract was used for the evaluation of total phenolic content and antioxidant capacity.

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The absorbance was detected at 765 nm. TPC were expressed as gallic acid equivalents (mg GAE $100g^{-1}$). Antioxidant properties were determined by the ABTS assay, according to Re at al. (1996) and through the evaluation of the free radical-scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical. Results were expressed as Trolox equivalent antioxidant capacity and as percentage of inhibition of the ABTS and DPPH radical.

2.4. Determination of minimum inhibitory concentration (MIC) of honey

The minimum inhibitory concentration was evaluated by the microdilution method with resazurin as an indicator (CLSI, 2012). *Staphylococcus aureus, Rhodococcus equi, Listeria monocytogenes* and *Seratia marcescens* were used in this study.

2.5. Statistical analysis

Statistical data were analyzed by one-way ANOVA using the statistical package Statistics 12 (StatSoft, Inc. 2014).

3. Results and discussions

3.1. Physico-chemical parameters

3.1.1. Water content and dry matter in honey

Water content is a quality parameter that is important for the durability of honey. It depends on many factors, such as beekeeping techniques, climatic conditions during the season, and the degree of maturity of honey reached in the honeycomb (Bogdanov et al., 2004). According to the Legislation on the Quality of Honey and Other Bee Products, the water content for all types of honey must not exceed 20% (Legislation, 2015). All tested samples met the criteria prescribed by the Legislation and had a water content less than 20%. The lowest value (18.5%) was measured in the sample of forest-meadow honey (I), and the highest (19.5%) in the sample of acacia honey (II). These results are correlated with the results of other authors (Getu and Birhan, 2014; Serin et al., 2018; Bloš et al., 2019). The values of the soluble dry matter content in the tested honey samples were in accordance with the Legislation, and the lowest value was determined for acacia honey (II) – 79%. (Table 2)

Table 2.
Contents of water, dry matter and HMF

Samples	Content of water %	Content of dry matter %	Content of HMF mg kg^{-1}
I	18.50	80.50	nd
II	19.50	79.00	1.09
III	19.00	79.50	1.79

Nd – not determined.

3.1.2. Content of HMF in honey

In the honey samples analyzed (Table 2), HMF was not detected in forest-meadow honey (I), while the highest content was detected in sunflower honey (1.79 mg kg^{-1}). The values obtained in this paper were in accordance with the values laid down by the Legislation (Legislation, 2015). Also, the content was significantly lower than 40 mg HMF·kg^{-1} which is the maximum value prescribed by Codex (2011). In addition to extremely low HMF content, forest-meadow honey also had the lowest water content (Table 2) compared to the other two tested honey samples; therefore, according to the Codex Alimentarius standard, it was defined as the freshest of all honey samples analyzed. Forest-meadow honey was from 2020. The content of HMF is a measure of honey freshness. It cannot be found in fresh foods (Mendešević, 2014). It is an indicator of honey quality and degree of deterioration. Dehydration of hexoses in the presence of acids, as well as the Maillard reaction, can result in the formation of the cyclic aldehyde HMF. HMF content depends on the type of honey, acid and water content, pH value, temperature and heating time, and storage conditions. Heat treatment and storage in inadequate conditions can cause spoilage and deterioration of honey (Rodriguez et al., 2019).

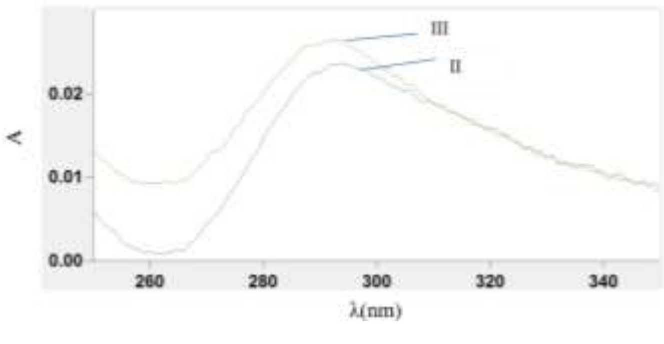

Figure 1. UV spectrum of samples: II – acacia honey, III – sunflower honey

3.2. Content of total phenols in honey

Based on the obtained results (Table 3), it was determined that the content of phenols (90.96 mg GAE 100g^{-1}) was highest in forest-meadow honey (I), and the lowest in acacia (15.75 mg GAE 100g^{-1}) and sunflower honey (19.53 mg GAE 100g^{-1}).

Table 3.
The content of phenolics and antioxidative activity in samples

Samples	Total phenolic content g GAE 100 g^{-1}	DPPH %	ABTS %	ABTS mmol Trolox 100 g^{-1}
I	$90.96\pm5.18_a$	$26.32\pm1.26_a$	$35.2\pm2.96_a$	0.4911
II	$15.75\pm0.66_b$	$7.28\pm0.15_b$	$11.76\pm0_b$	0.0932
III	$19.53\pm1.15_b$	$8.44\pm0.07_b$	$12.07\pm0.15_b$	0.1039

Means of triplicate determination (for phenolic content, DPPH and ABTS % inhibition). Means without the same superscripts within each column are significantly different ($P < 0.05$).

The results were in agreement with the results (Saxena et al., 2010; Atanacković-Krstonošić et al., 2019). The obtained data showed that, expectedly, forest-meadow honey, which was also the darkest, unlike the other two honey samples analyzed, had the highest content of total phenols, while acacia honey, being the lightest, had the lowest content of total phenols. Given the significant influence of phenols on honey color, according to the literature data, a correlation was found between honey color and total phenol content (Brudzynski and Miotto, 2011). Dark honey has a higher total phenol content than light honey (Brudzynski and Miotto, 2011). The obtained results are in agreement with the results of Bertoncelj et al. (2007).

3.3. Antioxidant activity

The antioxidant activity of the honey samples analyzed was determined by DPPH and ABTS methods. The highest antioxidant activity (26.32%) was measured in forest-meadow honey (I) and the lowest (II) in acacia honey (7.28, Table 3), whereas samples II and III showed no significant difference ($P< 0.05$). The highest antioxidant activity in forest-meadow honey was confirmed by the ABTS method (Table 3). The weakest antioxidant activity of acacia and sunflower honey obtained by DPPH and ABTS methods was caused by the low phenol content (Table 3). The results of antioxidant activity, expressed as % inhibition, by both DPPH and ABTS-methods, showed that forest-meadow honey had the strongest antioxidant properties (26.32 and 35.2%, respectively, Tables 3). It should be borne in mind that forest-meadow honey was from 2020, while acacia and sunflower honey were from 2018, and these two samples were crystallized. This affected the antioxidant potential of these samples, because the antioxidant properties of honey depend on the botanical and geographical origin, but also on the time of storage, since antioxidant properties decrease during storage. The results for honey samples from Slovenia regarding their antioxidant activity as measured by the DPPH method (Bertoncelj et al., 2007) revealed the highest antioxidant activity in forest honey, and the lowest in acacia honey. Similar results were reported by Atanacković-Krstonošić et al. (2019). The correlations between antioxidant activity and total phenol concentration in honeys were confirmed from Romania (Al et al., 2009), Italy (Rosa et al., 2011) and Poland (Kus et al., 2014). In Serbia, Gorjanović et al. (2013) and Savatović et al. (2011) determined a high correlation between TPC and antioxidant potential in an analysis of a few Serbian honeys using DPPH methods. Milivojević et al. (2010) and Đurović et al. (2021) found a correlation between phenol content and antioxidant activity. Blasa et al. (2006) and Salonen et al. (2017) argued that light-colored honey possessed lower antioxidant activity than darker colored honey, an observation that seems to be accurate for this study.

3.4. Minimum inhibitory concentration (MIC)

Table 4 shows the values of minimum inhibitory concentrations (MIC) for the honey samples analyzed, expressed as percentages. MIC is the lowest concentration of honey that stops the growth of bacteria in a suspension (Gobin et al., 2014). The sample with the lowest concentration has the highest antibacterial activity; in this case it was the sample of forest-meadow honey (I). As phenolic compounds affect the antimicrobial activity of honey, honey with a higher phenol content also has a higher antimicrobial activity (Escuredo, 2012), which was shown in this study (Table 3). Forest-meadow honey had the highest phenol content in relation to the other honey samples (Table 3); therefore, it showed the highest antibacterial activity and had an inhibitory effect at concentrations of 6.25% against the tested bacteria. The lowest antibacterial activity of all honey samples analyzed was exhibited by acacia honey, whose 25% solutions inhibited the growth of *Staphylococcus aureus, Seratia marcescens, Rhodococcus equi* and *Listeria monocytogenes.* As shown, antibacterial activity depends on the type of honey. It is possible that during the storage period of almost 3 years (for honey from 2018, samples II and III), there were changes and decreases in the content of certain components with antibacterial properties, which resulted in reduced antibacterial activity.

Table 4.
Values of minimum inhibitory concentrations (MIC) of honey (%)

Sample	*Staphylococcus aureus*	*Seratia marcescens*	*Rhodococcus equi*	*Listeria monocytogenes*
I	6.25	6.25	6.25	6.25
II	25	25	25	25
III	12.5	12.5	12.5	12.5

4. Conclusions

In this paper, the physico-chemical parameters, antioxidant properties and antimicrobial activity of three types of honey originating from Serbia were examined. The physico-chemical parameters of the honey samples analyzed had values that were within the limits laid down in the Codex Alimentarius standards and the Legislation on Quality of Honey and Other Bee Products. The water content ranged from 18.5 to 19.5%, and the soluble dry matter content varied between 79.0 and 80.5%. The HMF content was not detected in the sample of forest-meadow honey, while the highest content was detected in the sample of sunflower honey (1.79 mg kg^{-1}), but it was significantly below the values set by the Codex Alimentarius (40 mg kg^{-1}). The highest antioxidant activity, as measured by DPPH and ABTS methods, and a high content of total phenols were measured in forest-meadow honey, followed by sunflower honey, while acacia honey had the lowest antioxidant activity and the lowest content of total phenols. Forest-meadow honey showed the best antibacterial activity compared to the other two honey samples, and acted at the lowest concentration, 6.25%. Acacia honey showed the lowest antimicrobial activity towards the tested groups of microorganisms.

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