# PHYSICOCHEMICAL CHARACTERISTICS OF ACACIA AND MEADOW HONEY FROM DIFFERENT REGIONS OF THE REPUBLIC OF SRPSKA/BOSNIA AND HERZEGOVINA WITH AN EMPHASIS ON THE ENVIRONMENT OF BEEKEEPING ZONES

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**Abstract:** Honey is a thick, sweet, syrupy substance, the product of the honey-bee, *Apis mellifera L.*, obtained from the collected fruit juices and other, processed in the stomach of bees and is a pure product with no additives of any other substance. The paper present physical-chemical analysis for the following parameters, performed on 20 honey samples: sugar content, sucrose content, moisture content, free acidity, electrical conductivity, mineral content, the content of HMF and content of matter insoluble in water. As important indicators of the environment, this paper emphasises the analyses on the presents of antibiotic residues in two types of honey. The importance of establishing these indicators is to protect nature, which is the basis of agricultural development in the Republic of Srpska. The work suggests that the described bee region presents qualitative honey produced by using the natural resources of a designated area as an economic sector for the survival and development of those parts of the Republic of Srpska.

Keywords: physicochemical characteristics, honey, antibiotic, environment

## Introduction

Honey as a primary product of the honey-bee, is a natural product, variable not only in colour, taste and smell but also in its chemical composition and depends on the ecological environment and the vegetation of the honey plants (*Bogdanov*, 2009). Considering the number of possible sources of honey grazing, it is understandable that there is no completely identical honey from different localities (*Ivanovic et al., 2015; Matović et al., 2018*). While this is an under-clarified issue from the market demand perspective, at the same time, this diversity makes it possible to choose the type of honey with the best features. Scientifically, this

variability can be the result of various factors, but in most cases, it is related to the botanical origin of honey and agroecological conditions (*Kaškoniené and Venskutonis, 2010*).

In the European Union, the results of the analysis of honey on the content of heavy metals and pesticide residues serve as an indicator of the degree of environmental contamination, that is, the state of product quality of the area (*Al-Waili et al.*, 2012).

From a chemical point of view, honey is a highly concentrated solution of a complex sugar mixture (*Codex Alimentarius, 2001*). Its composition depends largely on the plant species from which the nectar or honeydew was collected, as well as the environmental and climatic conditions. In addition to sugar, honey has a wide range of less present compounds, many of which including polyphenols, have anti-oxidant effects. Honey is considered a potential complete food, in terms of dietary standards, as a natural product, rich in simple and highly assimilable sugars, enzymes, amino and organic acids, vitamins, volatile oils, minerals, carotenoids, phenolic acids and flavonoids (*Belitz et al., 2009; Milojković Opsenica et al., 2015*).

The Codex Alimentarius, the EU Legislation and the National Standards of the States prescribe the authenticity of honey. Authenticity in terms of production and authenticity in terms of geographical origin and botanical originality are two major aspects of the general authenticity of honey (*Lazarević, 2016*).

The presence of established honey types will be an indicator of the variety and originality of honey, which will result in the separation of monofloral from polyfloral honey, as well as suppression of counterfeiting. The assumption is that this established network of the most important grazing areas of the Republic of Srpska lays the foundation for the determination of ecologically clean zones in our area. This paper points to the possibilities of economic and agricultural justification in the production of ecologically quality honey in 4 different regions. The research should point to the environment in which we are dealing with agriculture, ie the possibilities of sustainability of our products, through quality, in the world market.

### **Materials and Methods**

Samples were collected during 2011, at the end of honey collection season. An investigation of samples was done at the Institute for public health of the Republic of Srpska (RS), with a place in Banja Luka and the Institute for hygiene and technology of meat in Serbia, with a place in Belgrade. A total of 20 samples of different species of honey (acacia and meadow honey) was taken from 4 different parts of RS. From Hercegovina region were collected 5 samples of meadow honey, the same was for Romanija region, for both, Doboj and Krajina were taken 5 samples of acacia honey. For a determinate of botanical region of honeys, we used their declarations, sensory characteristic and geographical origins.

Samples of honey were kept in their original packages at 20-21°C in the dark before laboratory analysis. A honey investigation was done using the International Honey Commission (2009), recommended standard methods of official analyses methods from the Association of Official Analytical Chemists (*AOAC, 1990*). Chemical analyses measured the following perimeters: total inverted sugars, reducing sugars, sucrose, water, acidity, electrical conductivity, mineral materials, hydroxymethylfurfural (HMF) and water-insoluble content.

The content of sugars was measured by aperture Meopta (Meopta, Germany), testing was applied with refractometric detection (HPLC-RI) and automatic compensation of temperature between 10-30 °C (*Rybak-Chmielewska*, 2007), concentration of sugar is presented in the form of percentage (%).

Water content was estimated from the refractive index measured with refractometric detection (HPLC-RI) by aperture Meopta (Meopta, Germany). testing was applied and automatic compensation of temperature on 20°C, after waiting for 6 min for equilibrium and results were converted in the form of a percentage (%). Form determination of free acidity was used via the titrimetric methodology with 0.1 of sodium hydroxide. The solution was titrated with 0.05 NaOH and parameters are presented in mEq/kg. Electrical conductivity (EC) measured at 20°C in a 20% of honey solution in water with a KIT consort conductometer (Wissenschaftlich-Technische Werkstätten. Germany) and expressed as mS/cm. Mineral materials and water-insoluble content were measured by gravimetric method determinated by Labmaster-a apparatus (Novasina, Switzerland) on 25 °C, results are expressed in precent (%).

The HMF (hydroxymethylfurfural) content was done on a HPLC apparatus/method (Hewlett Packard, USA). A detailed procedure of HMF determination described by *Zappala et al.* (2005), parameters are expressed in mg/kg, according to the International Honey Commission (2009).

Both laboratories of Institutes have implemented accreditation by BAS-EN-ISO/IEC 17025-2006; LI-40-01, for good quality system results of physical – chemical analyses. Results were compared with Codex Alimentarius, BiH regulations (*Codex Alimentarius 2001, Rulebook BiH, 2009, 2011*) and European Council Directive 2001/1100/EC (2002).

For determination of antibiotic residues microbiological method "Modified method 4 plates" was used (Heitzman, 1994). Determination was done with a test MUVA PROFICIENCY TESTING apertures (Kempten, Germany). The main principle of the test is on detection of microorganism growth inhibition during the presence of antibiotics, test consist a layer of inoculated nutrient agar (*Apić et al., 2015*). In BiH, the use of antibiotic to prevent bacterial diseases is prohibited and accordingly no MRL (maximum residue limits) for antibiotic in honey, in this paper we use relevant provisions for of certain ordinances and prescribed methods for quality control (*Rulebook, BiH 2009*).

#### Statistical data processing

Data were analysed by one-way analysis of variance (effect of experimental group) using GLM procedures in the statistical software SPSS Statistics (version 17.0). Data are presented as LS means with respective standard errors. In case that F-test was significant (P<0.05) differences between samples were evaluated using the Tukey test.

### **Results and Discussion**

#### Physicochemical characteristics of acacia and meadow honey

Results of physicochemical characteristics of two types of honey were presented in table 1 and 2.

Type of honey	Acacia	Acacia	p – Value	
Region	Krajina	Doboj		
Number of honeys	5	5		
Total inverted sugars (%)	$76.25 \pm 1.32$	77.77 ± 1.14	0.42	
Reducing sugars (%)	$73.10 \pm 1.26$	$73.55\pm0.29$	0.74	
Sucrose content (%)	$2.97\pm0.29$	$4.05 \pm 1.15$	0.40	
Water (%)	$16.50\pm0.58$	$16.62\pm0.50$	0.87	
Free acidity (milieqv kis/g)	$11.70 \pm 1.51$	$9.62\pm0.62$	0.25	
Electrical Conductivity (mS/cm)	$0.28\pm0.04$	$0.23 \pm 0.01$	0.28	
Mineral materials (%)	$0.04\pm0.00$	$0.04 \pm 0.01$	1.00	
HMF (mg)	$5.67 \pm 1.09$	$7.65 \pm 2.80$	0.53	
Water-insoluble content (%)	$0.01\pm0.00$	$0.01\pm0.00$	0.35	

Table 1. Results of physico-chemical	parameters	of	Acacia	honey	from	Krajina	and	Doboj
regions of RS (LS means ± SE).								

Carbohydrates are the main constituent of honey with content of 73-83%, so honey can be defined chemically as a saturated solution of sugar. It makes up more than 95% of the dry matter in honey, fructose and glucose monosaccharides about 85-95% of the total sugar content (*Lazarevic, 2016*). Statistically differences between the regions of acacia honey, for the content of total inverted sugars were not observed (Table 1). Environmental factors probably had no influence on a variation of redacted sugars in honey. Also, water and humidity as well as wind can affect the honey-bee and the physical and chemical properties of honey (*Semkiw et al., 2008*). Other authors report similar results (*Marghitas et al. 2009; Ciric et al., 2018; Dogo Mračević et al. 2019*). Furthermore, higher content of the invert is present in the Herzegovina region (Table 2), because the external environmental

factors (humidity, sunlight) provided good bee pasture. The weather conditions were ideal, the flowering period of the plants is much longer than in the Romani region. Also, the type of honey plants affects the sugar content of honey, in Herzegovina, honey plants with a higher sugar content (sage, chestnut) are more prevalent. Similar results were reported by *El Sohaimy at. al.*, (2015), where is explanted that content of sugar is indicator of ability for honey crystallisation. Parameters are in accordance with international standards established by the *Codex Alimentarius Commission (2001)* (with a level of not less than 60g/100g of reducing sugars in honey).

Table 2. Results of physico-chemical parameters of meadow honey from the Hercegovina and Romanija region of RS (LS means  $\pm$  SE).

Type of honey	Meadow honey	Meadow honey			
Region	Hercegovina	Romanija	p - Value		
Number of honey	5	5			
Total inverted sugars (%)	$78.50\pm0.94^{a}$	$70.76 \pm 2.49^{b}$	0.02		
Reduced sugars (%)	$75.12\pm1.41^{\rm a}$	$68.40 \pm 2.58^{b}$	0.05		
Sucrose content (%)	$3.25\pm0.70$	$2.30\pm0.34$	0.26		
Water (%)	$16.54\pm0.80$	$17.34\pm0.40$	0.40		
Free acidity (milieqv kis/g)	$23.76 \pm 1.95$	$28.76 \pm 1.98$	0.11		
Electrical Conductivity (mS/cm)	$0.62\pm0.04^{a}$	$0.77 \pm 0.04^{b}$	0.05		
Mineral materials (%)	$0.22\pm0.07$	$0.22\pm0.01$	0.94		
HMF (mg)	$7.40 \pm 1.49$	$7.40 \pm 4.54$	1.00		
Water-insoluble content (%)	$0.02\pm0.01$	$0.02\pm0.01$	1.00		

Notes: <sup>a,b</sup>Statistically significant differences between the groups are denoted by different superscript letters.

The allowed water content in honey, according to Codex Alimentarius and BiH regulations (*Codex Alimentarius 2001, Rulebook, 2011*) should not be more than 20%.

The water content of honey determines its stability and quality (*Abdulkhaliq and Swaileh, 2017*). The most suitable would be to store honey containing less than 18% water without a risk of fermentation, although the ordinance provides for up to 20% (*Bilić, 2013*). Water content is the most important parameter of honey quality, as it plays a key role in preserving stability and preventing microbial processes that result in fermentation and the spoilage of honey (*Lazarevic et al., 2013*).

The water content of acacia honey of the Herzegovina region is 16.54 % and in the region of Romania 17.34, 16,50 % for the meadow honey in Krajina and 16.25 %

in the Doboj region. Results are in accordance with another published research (*Mirjanić and Mladenović, 2012; El Sohaimy et al., 2015; Vranić et al., 2017; Ciric et al., 2018*).

The water content affects the significant physical properties of honey, such as crystallisation and viscosity (*Bilić*, 2013). The higher content of water in honey, follows a fermentation process caused by the osmophilic yeast (*Saccharomyces spp.*), the fermentation process leads to changes in the chemical composition and organoleptic properties of honey (because fermentation of glucose and fructose produces ethanol and carbon dioxide) which creates a foam on the surface of the product (*Isengard and Daniela 2003; Gallina et al.*, 2010).

Very important factors for the classification of honey analyses according to their geographical origin are water content, electrical conductivity and free acidity (*Ciric et al. 2018*).

Differences between 4 regions of two types of honey were not observed for free acidity. Results are according to the Codex Alimentarius and BiH regulations (not more than 50 meq/kg). While electrical conductivity was only statistically different between the Hercegovina and Romanija regions for meadow honey (p - 0.050). Some authors state that EC is in relationship with measurements of ash and acid (El Sohaimy et al. 2015). In our research free acids were higher in meadow honey compared to acacia honey, which agrees with other published papers (Cirić et al. 2018, Vranić et al., 2017). The hydroxymethylfurfural parameter in honey represents damage caused by heating, it is usually absent in fresh and untreated honey (Matović et al., 2018). Our results agree with maximum limited concentration of 40 mg/kg, recommended by the European Union (EU Directive 110/2001). The HMF between two groups of meadow honey was 7.40 mg/kg. between the acacia range from 54.67 to 7.65 mg/kg. Results indicated that honey from different regions was of a good quality; results are not in agreement with the Ciric et al (2018) research. Similar results were obtained for acacia honey, published by Vranić et al., (2017). Minerals are present in honey in very small quantities, mostly potassium. In addition to potassium, honey contains other chemical elements such as sodium, calcium, magnesium, iron, copper, manganese, aluminum, molybdenum, cobalt, zinc, chlorine, phosphorus, sulfur, etc (Bilić, 2013; Czipa et al., 2019). Average values do not differ statistically significantly between measured groups. The same results were obtained for acacia and linden honey in a research in Serbia (Vranić et al., 2017, Matović et al., 2018). A research published by Grujić and Komić (2012) states that with a different content of minerals materials can prove the botanical origin of different species of honey.

The maximum permissible reference value for the content of substances insoluble in water for meadow and acacia honey is 0.1 g /100g, the values obtained in all four beekeeping regions vary between 0.010 g/100g and 0.028 g100/g, which is in accordance with the criteria for physico-chemical parameters of honey established by regulations (*Official Gazette BiH*, 2009, 2011). Results are in accordance with results published for research in the Balkan region (*Vranić et al., 2017, Ciric et al., 2018. Matović et al., 2018*).

Table 3. shows the results of the presence of antibiotics in honey, it demonstrates that antibiotic residues were not found in all 20 samples honey. The results are in accordance with SFRJ regulation for the pesticides residues, metals and metalloids, chemotherapeutics. anabolic and other substances that may be present in foods (*Rulebook SFRJ*, 2002).

Antibiotics residues	Meadow honey	Acacia honey		
Units	I	Prescribed value		
Number of honeys	10	10	varue	
Amoxycillin	not determined	not determined	not allowed	
Ampicillin	not determined	not determined	not allowed	
Benzil penicilin G	not determined	not determined	not allowed	
Chlortetracycline	not determined	not determined	not allowed	
Cloxacillin	not determined	not determined	not allowed	
Dicloxacillin	not determined	not determined	not allowed	
Dihydrostreptomycin	not determined	not determined	not allowed	
Doxicycline	not determined	not determined	not allowed	
Erythromycin	not determined	not determined	not allowed	
Gentamycin	not determined	not determined	not allowed	
Kanamycin	not determined	not determined	not allowed	
Lincomycin	not determined	not determined	not allowed	
Neomycin	not determined	not determined	not allowed	
Oxacillin	not determined	not determined	not allowed	
Oxytetracycline	not determined	not determined	not allowed	
Spectinomycin	not determined	not determined	not allowed	
Tetracycline	not determined	not determined	not allowed	
Tylosin	not determined	not determined	not allowed	

Table 3. Results of laboratory test of honey samples for the presence of residues of antibiotic

In the honey and its product, antibiotics are often used for veterinary setting, such as streptomycin, sulfonamide, and chloramphenicol (*Al-Waili et al., 2012*). Maximum residue limits were not allowed for honey and its products by regulation of European Union (*EC Directive 1990, 2009 and 2010*). High levels of antibiotics in honey exported from India to the EU and the US have been reported by agricultural food producers to the Development Agency from 2005 onwards (*Solomon et al., 2006*). In 2006, about 14% of samples were contaminated with

tetracyclines and between 2007-2008 about 28% of the samples were contaminated with the same antibiotic. In the period between 2009-2010, 462 honey samples were analysed, 29.2% of the samples had more than the prescribed limit for the presence of antibiotics. In 2000-2001, streptomycin was detected in 4/248 samples, tetracycline in 2/72 samples, and sulfonamides in 1/72 samples (*Solomon et al., 2006, Forsgren, 2010*). Our presented results are not in accordance with published results by authors *Ortelli et al. (2004), Saridaki-Papakonstadinou et al., (2006) and Johnson et al., (2010)*, where a large number of studies cited in the last 20 years have identified antibiotics in wax, pollen, bees and honey. With these presented results, we can confirm ecological clean beekeeping zones in the RS.

### Conclusion

An examination of the physico-chemical parameters has determined a fulfilment of requirements regarding the satisfaction of the quality of monofloral and polyfloral honey of the Republic of Srpska in accordance with domestic and European Regulations. Counterfeits in the work have not been proven. It is obtained that mountain honey in comprising with honey from lowland plains, was of a higher quality. The microbiological safety of the tested honey species (presence of antibiotics) in the Republika Srpska region was determined.

The results of the analyses presented in this paper, confirm that two types of honey from different parts of the Republika Srpska, due to the diversity of honey and medicinal plants, climatic conditions and that type of production represents a great potential for the investigated areas and deserves a great commitment from competent institutions.

# Fizičko-hemijske karakteristike bagremovog i livadskog meda iz različitih područja Republike Srpske / Bosne i Hercegovine s naglaskom na okruženje pčelarskih zona

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## Rezime

Med je gusta, slatka, sirupasta supstanca, proizvod medonosne pčele, *Apis mellifera L.*, dobijen od prikupljenih voćnih sokova i drugog, prerađenog u stomaku pčela i čisti je proizvod bez dodataka bilo koje druge supstance. Fizičkohemijska analiza za sledeće parametre izvedena je na 20 uzoraka meda: sadržaj

šećera, saharoze, sadržaj vlage, slobodna kiselina, električna provodljivost, sadržaj minerala, sadržaj HMF i materija nerastvorljivih u vodi. Kao važan indikator životne sredine, u ovom radu se ističu analize zastupljenosti ostataka antibiotika u dve vrste meda. Važnost uspostavljanja ovih pokazatelja je zaštita prirode, što je osnova razvoja poljoprivrede u Republici Srpskoj. Rad sugeriše da opisani pčelinji kraj predstavlja kvalitativni med proizveden korišćenjem prirodnih resursa određenog područja, kao ekonomskog sektora za opstanak i razvoj tih delova Republike Srpske.

Ključne reči: fizičko-hemijske karakteristike, med, antibiotici, životna sredina

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