Microbiological and physico-chemical quality of honey in Bosnia and Herzegovina

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Abstract

The aim of the study was to investigate the microbiological and physico-chemical quality of honey samples, sampled for the event "Honey Days in FB&H 2020", which tested a total of 33 samples of different honey types from different geographical areas of Bosnia and Herzegovina (B&H). The aim of this study was to evaluate the relationship between physico-chemical properties and microbiological properties of the tested honey to assess quality. The submitted samples originated from both administrative units of B&H, namely from the continental, sub-Mediterranean and Mediterranean part, covering more than 60% of the country's territory. Of the total number of analysed samples (n=33), nine samples (29.7%) did not meet the requirements of physico-chemical and microbiological parameters of the test. The requirements of one or more quality parameters according to the national Ordinance on methods for the control of honey and other bee products in B&H (Anonymous, 2009) were not met by five samples (16.5%). Four samples (13.2%) did not meet the microbiological quality, as they exceeded the permitted number of yeasts and moulds. In five samples (16.5%), the presence of microorganisms was recorded within the tolerance limits, while *Enterobacteriaceae* and sulphite-reducing *clostridia* were not isolated and were below the detection limit in all samples. Honey samples in which the results were found to be unsatisfactory for physico-chemical parameters and microbiological parameters cannot be correlated.

Key words: *honey; microbiological analysis; physico-chemical analysis; honey quality; Bosnia and Herzegovina*

Introduction

Honey is one of several Bosnia and Herzegovina (B&H) products of animal origin that are licensed for export to the EU market, though the export opportunities have not yet been fully exploited. One of the main reasons why small quantities of quality honey from B&H are delivered to the EU market is the limited production, that primarily covers the domestic market. According

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to the available data, the average honey production in B&H over a 10-year period was 3230 tonnes, and the average yield of honey per hive is about 9 kg (Anonymous, 2017). The European Union (EU) produces 250,000 tonnes of honey per year. However, this amount of honey covers only 60% of the market demand, with an average honey consumption of 0.9 kg per capita per year. The remaining 40% of the required amount of honey is imported. In the EU, honey is one of the top 10 food products that are most often counterfeited. Counterfeit honey poses a risk to public health, and therefore it is of great importance to constantly conduct physico-chemical and other analyses of honey quality (Franić and Branica, 2019).

The most common ingredients in honey are carbohydrates, among which fructose and glucose predominate. Substances such as organic acids, enzymes and solid particles enter honey during its formation. Carbohydrates are the most common ingredient in honey, with a share of 73 to 83%. The water content in honey usually ranges between 14.5 and 18.5%. Values greater than these may indicate fermentation. However, some types of honey naturally contain a higher proportion of water (chestnut from 17 to 19%, heather up to 21%). The remaining components of honey, other than carbohydrates and water, make up less than 1.5% of honey, and these are organic acids, nitric substances, minerals and some vitamins (Bogdanov et al., 1999). Rončević et al. (2018) state that the water content in honey is one of the most important parameters of honey quality, as an indicator of its maturity and stability, though it is not used for determining botanical origin (Svečnjak et al., 2015), and that selected physico-chemical parameters (water content, electrical conductivity and pH value) may indicate the quality of honey in accordance with European criteria.

The natural ingredients present in honey are responsible for a wide range of antimicrobial (antibacterial, antifungal and antiviral) activity. The presence of specific substances (such as phenols, flavonoids, aromatic acids), acidity, osmolality and enzymatic production of hydrogen peroxide in honey, inhibit the growth and reproduction of pathogenic bacteria and food spoilage agents (Kiš et al., 2019). Honey has two basic groups of mechanisms by which it exerts antibacterial activity. The first group includes mechanisms of antibacterial activity based on its physico-chemical properties (osmolality, viscosity, pH value or acidity). The second group of mechanisms of antibacterial activity of honey is based on the chemicals present in it (hydrogen peroxide, methylglyoxal and antimicrobial peptide bee defensin-1). Due to the influence of various factors on the antibacterial properties of honey, it is not possible to accurately predict how much antibacterial activity a honey will possess, so it is necessary to test the antimicrobial properties of honey before possible therapeutic application (Gobin et al., 2014).

Due to its complex chemical composition, honey is an unfavourable medium for the growth of microorganisms. High sugar concentration, low pH value, low water activity, the presence of hydrogen peroxide and other specific substances in honey have an inhibitory effect on the growth and reproduction of microorganisms in honey. The microorganisms that can survive in these conditions in honey are primarily yeasts and moulds and sporoform bacteria. Natural honey shows great variability in antimicrobial activity against pathogenic bacteria, because the composition of active ingredients in plants depends on various factors, especially on plant cultivar and chemotype, and on climatic conditions (Gradvol et al., 2015).

Honey can be contaminated with microorganisms through primary (pollen, bee digestion, dust, air, soil

and nectar) and secondary (after harvest: packaging, handling, cross-contamination, equipment and storage) sources (Borum and Gunes, 2018). The most common genera are *Bacillus, Clostridium, Penicillium, Mucor, Saccharomyces, Schizosaccharomyces,* and *Torula*. Sulphite-reducing clostridia is an indicator microorganism, and its presence in honey is evidence of contamination. The presence of *Clostridium* spp. spores is particularly dangerous for infants and young children, while paediatric botulism is caused by the consumption of honey contaminated with *C. botulinum* (Erkan et al., 2015). The increase in the number of yeasts in honey cannot be related to the type of honey, despite differences in pH value and acidity (Gomes et al., 2009). Aerobic mesophilic bacteria are part of the normal flora of the digestive system of bees (Kacániová et al., 2009).

Research on the health safety of honey is focused primarily on bacterial contamination. As a source of free amino acids, sugars and minerals, honey is a suitable medium for the development of yeasts and moulds, especially due to improper handling during production and unfavourable storage conditions. (Kiš et al., 2019).

The quality of honey is determined primarily by its botanical properties, and then by its organoleptic, physico-chemical and microbiological properties. The purpose of this study was to evaluate the relationship between physico-chemical properties and microbiological properties of the tested honey in order to assess its quality.

Material and methods

During 2020, 33 samples among all the samples entered in the event "Honey Days in FB&H 2020" were taken for microbiological and physico-chemical analysis. The samples originated from beekeepers of registered farms in more than 60% of the territory of B&H (continental, sub-Mediterranean and Mediterranean areas). One part of the honey samples is from the northern continental part of B&H, from the Danube Basin, *i.e.* the Una, Bosnia and Drina Rivers, which are rich in forest pastures and flora (chestnut, acacia, mountain flowers, dandelion, clover, continental fruit, raspberry, blackberry, cherry). The second part of the sample originated from the southern sub-Mediterranean and Mediterranean region of B&H, *i.e.* the Adriatic Sea Basin, i.e. the Rakitnica, Neretva and Trebižat River catchments, which are rich in grazing with honey-bearing Mediterranean nectars characteristic for this part of B&H (almond, thorn, sage and heather). The samples were stored in sterile glass jars at room temperature until analysis.

Microbiological analysis: Microbiological analysis of honey samples was performed according to the B&H guidelines on microbiological criteria for food (Anonymous, 2013). Microbiological analyses included aerobic mesophilic bacteria, *Enterobacteriaceae*, sulphite-reducing clostridia, and yeasts and moulds. The microbiological criterion was taken as one sample without subsamples. Standard ISO microbiological methods of growing on rootstocks were used for counting and identification of microorganisms in honey. Analyses were performed in the Federal Institute of Agriculture Sarajevo and the Federal Agro-Mediterranean Institute Mostar, which are accredited according to ISO standard 17025 (Tables 1 and 2).

Physico-chemical analysis: Physico-chemical tests were performed according to the methods from the Ordinance on methods for control of honey and other bee products in B&H (Anonymous, 2009). Analyses were performed at the Federal Institute of Agriculture Sarajevo and the Federal Agro-Mediterranean Institute Mostar, which are accredited according to ISO standard 17025 (Table 3).

Table 1. Criteria of recommended microorganisms tested in honey according to the guidelines for microbiological criteria for food in B&H (Anonymous, 2013)

	Microorganisms	Criteria
Recommended	Aerobic mesophilic bacteria	10^3-10^4 cfu/g
	Enterobacteriaceae	10-10^2 cfu/g
	Sulphite-reducing clostridia	10 cfu/g
	Yeasts and moulds	10-10^2 cfu/g

cfu/g - (colony forming unit per gram)

Table 2. Microbiological methods of isolation and identification

Microorganisms	Nutrient medium	Incubation conditions	Method
Aerobic mesophilic bacteria	Plate Count agar (PCA-agar)	30°C during 72 h	BAS EN ISO 4833-1:2014
Enterobacteriaceae	Violet Red Bile Glucose agar (VRBG-agar)	37°C during 24 ± 2 h	BAS EN ISO 21528-2:2018
Sulphite-reducing clostridia	Iron sulphate agar (ISA-agar)	37°C during 24-48 h	BAS EN ISO 1513:2008
Yeasts and moulds	Dichloran 18 % mass fraction glycerol agar (DG 18 agar)	25°C during 7 days	BAS EN ISO 21527-2:2009

Table 3. Criteria for honey composition according to the Ordinance on methods for control of honey and other bee products in B&H (Anonymous, 2009).

TYPE	Referent value
MOISTURE CONTENT In general (applies to all types of honey except those listed below)	not more than 20%
ELECTRICAL CONDUCTIVITY Honey not listed in the row below and mixtures of these types of honey	not more than 0.8 mS/cm
ELECTRICAL CONDUCTIVITY Honeysuckle and chestnut honey and their mixtures, with exceptions (exceptions: plantain or strawberry, bell heather, eucalyptus, heather, tea tree)	not less than 0.8 mS/cm
Free acids In general (applies to all types of honey except those listed below)	not more than 50 milieqv ac./1000 g
Sucrose content In general (applies to all types of honey except those listed below)	not more than 5 g/100 g
Sucrose content Acacia, alfalfa, Menzien bank, red gum, citrus	not more than 10 g/100 g

Table 4. Results of microbiological research parameters

Order no:	Honey type	Aerobic mesophilic bacteria / g	*Enterobacteriaceae* / g	Sulphite-reducing clostridia / g	Yeasts and moulds / g
		Microbiological criteria:			
		10^3-10^4 cfu/g	10-10^2 cfu/g	10 cfu/g	10-10^2 cfu/g
1.	Floral 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
2.	Mixed 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
3.	Honeydew 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
4.	Meadow 1	4x10^2 cfu/g	<10 cfu/g	<10 cfu/g	5x10^3cfu/g
5.	Meadow 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	9x10^2 cfu/g
6.	Floral 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
7.	Floral 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
8.	Meadow 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
9.	Mixed 2	4.8x10^3 cfu/g	<10 cfu/g	<10 cfu/g	1.1x10^3 cfu/g
10	Mixed 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
11.	Sage 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
12.	Heather 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
13.	Chestnut 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
14.	Floral 4	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
15.	Meadow 4	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
16.	Heather 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
17.	Floral 5	9.3x10^3 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
18.	Mountain meadow 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
19.	Mountain meadow 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
20.	Mountain meadow 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
21.	Acacia 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
22.	Heather 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
23.	Meadow 5	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
24.	Floral 6	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
25.	Acacia 2	1x10^2 cfu/g	<10 cfu/g	<10 cfu/g	1x10^2 cfu/g
26.	Chestnut 2	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
27.	Chestnut 3	1x10^2 cfu/g	<10 cfu/g	<10 cfu/g	1x10^2 cfu/g
28.	Chestnut 4	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
29.	Chestnut 5	1x10^3 cfu/g	<10 cfu/g	<10 cfu/g	1.4x10^3 cfu/g
30.	Floral 7	<10 cfu/g	<10 cfu/g	<10 cfu/g	1x10^2 cfu/g
31.	Meadow 6	2.5x10^2 cfu/g	<10 cfu/g	<10 cfu/g	1x10^2 cfu/g
32.	Meadow 7	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
33.	Meadow 8	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g

Determination of sugar was done according to the Luff-Schoorl method. The method is based on the principle that under certain conditions a reducing sugar (natural invert) converts Cu^{2+} ions into Cu^{+} ions.

The unused amount of Cu^{2+} ions is re-titrated with standard sodium thiosulfate solution. From the difference between the consumption for the blank probe and the sample, the amount of sugar can be seen from the table, which shows the relationship between the consumption of the reagent and the amount of sugar.

Determination of sucrose was performed according to the Ordinance on methods for control of honey and other bee products in B&H (Anonymous, 2009) Annex II, section D, and is calculated as the difference in the content of reducing sugars before and after hydrolysis, multiplied by a factor of 0.95. Free acidity was determined by titration with a standard solution of sodium hydroxide with phenolphthalein as an indicator. The moisture content was determined gravimetrically by drying at 100–105°C. The electrical conductivity at 20°C was determined using a conductometer using an aqueous solution of honey (the amount of honey equivalent to 20 g dry matter of honey dissolved in 100 mL distilled water).

Results

a) Microbiological testing

The results are presented in such a way that the finding for aerobic mesophilic bacteria in honey in accordance with the provisions of the regulations is presented as negative (<10 cfu/g), acceptable (up to 10^3-10^4 cfu/g) or unsatisfactory (>10^4 cfu/g), and yeasts and moulds as negative (<10 cfu/g), acceptable (10-10^2 cfu/g) and unsatisfactory (>10^2 cfu/g). *Enterobacteriaceae* and sulphite-reducing clostridia were not isolated (Table 4).

Of the total of 33 examined honey samples, four samples (13.2%) did not meet the microbiological quality (sample no. 4 and 5 declared as meadow honey, sample no. 9 mixed honey and sample no. 29 chestnut honey). These honey samples had yeasts and moulds that exceeded the allowed 10^2 cfu/g, according to the recommendations from the Guidelines on Microbiological Criteria for Food in B&H, which are adapted to EU regulations and the Directive on microbiological criteria for food (2075/2013). Aerobic mesophilic bacteria were found in sample nos. 4, 9, 17 and 31, though within the permissible limit, up to 10^4 cfu/g. In five samples (16.5%), the presence of all microorganisms listed by the Guideline criteria was recorded, but less than or within the permitted limits (sample nos. 4, 25, 27, 30 and 31). Of the total number of analysed samples, the presence of yeasts and moulds within the permitted range of 10 to 10^2 cfu/g was found in four (13.2%) samples (nos. 25, 27, 30 and 31), and aerobic mesophilic bacteria in the range 10 to 10^2 cfu/g were found in two (6.6%) samples (no. 25 and 27). Aerobic mesophilic bacteria exceeding 10^2 cfu/g were recorded in two (6.6%) samples (nos. 4 and 31).

b) Physico-chemical testing

The results of physico-chemical testing of honey samples are presented in Tables 5 and 6.

Table 5. Average results of parameters of physico-chemical composition of honey

Parameter		Value
Water content, %		17.81
Acidity, milieqv ac./1000 g		30
Electrical conductivity, mS/cm		0.76
Sugars, %	Total	69.31
	Reducing	67.69
	Sucrose	1.57

The requirements for the physico-chemical quality of the analysed honey samples were not met by 5 (16.5%) of the total 33 samples, for one or more quality parameters (Table 6).

Table 6. Total results of parameters of the physico-chemical composition of honey

Order no.	Honey type	Sugars %			Water content (%)	Acidity (milieqv ac./1000 g)	Electrical conductivity mS/cm)
		Total	Reducing	Sucrose			
1.	Floral 1	64.05	63.45	0.60	16.10	39.00	1.18
2.	Mixed 1	64.84	64.27	0.60	17.20	47.04	1.04
3.	Honeydew 1	56.84	55.76	0.60	17.80	32.91	1.14
4.	Meadow 1	57.23	56.63	0.60	17.60	38.91	0.70
5.	Meadow 2	68.23	67.63	0.60	18.00	29.60	0.45
6.	Floral 2	68.77	68.17	0.60	18.67	37.76	0.28
7.	Floral 3	67.27	66.67	0.60	17.20	29.10	0.12
8.	Meadow 3	71.14	70.54	0.60	17.67	25.71	0.21
9.	Mixed 2	65.59	64.99	0.60	17.00	45.28	0.96
10.	Mixed 3	61.54	60.94	0.60	16.40	52.28	1.36
11.	Sage 1	73.24	72.64	0.60	16.50	36.40	0.55
12.	Heather 1	62.79	62.19	0.60	17.00	39.30	1.31
13.	Chestnut 1	71.46	69.84	1.62	16.40	21.52	0.98
14.	Floral 4	64.44	63.84	0.60	16.40	27.21	0.40
15.	Meadow 4	72.45	69.98	2.47	16.60	31.56	0.17
16.	Heather 2	66.88	65.32	1.56	15.20	24.21	0.19
17.	Floral 5	69.36	68.76	0.60	16.80	25.32	0.12
18.	Mountain meadow 1	71.14	69.11	2.03	18.35	31.00	0.83
19.	Mountain meadow 2	69.72	65.64	4.08	21.10	42.00	0.76
20.	Mountain meadow 3	74.96	74.46	0.50	18.95	34.00	0.31
21.	Acacia 1	75.45	74.94	0.51	18.12	22.00	0.31
22.	Heather 2	72.72	70.20	2.52	20.35	32.00	0.30
23.	Meadow 5	73.09	70.05	3.04	17.30	28.00	0.38
24.	Floral 6	72.67	71.70	0.97	18.76	38.00	0.57
25.	Acacia 2	76.69	75.14	1.55	15.35	14.00	0.17
26.	Chestnut 2	69.23	69.00	0.23	17.80	19.00	1.97
27.	Chestnut 3	72.94	71.40	1.54	18.40	22.00	2.04
28.	Chestnut 4	69.33	69.10	0.23	22.20	21.00	1.65
29.	Chestnut 5	70.83	70.32	0.51	19.10	20.00	1.90
30.	Floral 6	72.71	66.55	6.16	16.10	17.00	0.32
31.	Meadow 6	72.66	65.99	6.67	19.00	25.00	0.87
32.	Meadow 7	70.71	68.15	2.56	20.20	22.00	0.74
33.	Meadow 8	74.94	70.31	4.63	18.00	20.00	0.75

The average water content in all analysed types of honey was 17.81%, the acidity was 30 milieqv ac./1000 g, the electrical conductivity was 0.76 mS/cm and the total sugars were represented by 69.31%.

Discussion

The microorganisms detected in the tested honey samples have also been reported by other authors. In this study, the results are correlated with research conducted across Europe. Gradvol et al. (2015), tested 72 honey samples in Croatia, and did not detect *Enterobacteriaceae* and *Clostridium spp.*, while aerobic mesophilic bacteria, found in all types of honey, were within the acceptable limits, as in this study. Sinacori et al. (2014) tested 38 honey samples in Italy and only three were positive for the presence of yeasts and moulds, but not at concentrations higher than 10^2 cfu/g, as presented here. *Enterobacteriaceae* were found in only two samples, while clostridia was determined in 15 samples, with the highest estimated value of 0.92 MPN/g. Kiš et al. (2019) 16 honey samples in north-western Croatia, and found aerobic mesophilic bacteria and yeasts and moulds in 40% of the tested samples. The presence of aerobic mesophilic bacteria above the prescribed maximum concentration was found in 7.5% of samples, yeast in 25% of samples, and mould at concentrations of 10 to 10^2 cfu/g in 27.5% of samples, with yeast and mould contamination in 12.5% of samples. In Poland, the honey samples had concentrations of yeasts and moulds less than 10^2 cfu/g (Felsociova et al., 2012). In a large study in Poland on 245 samples of different types of Polish honey, the concentrations of tested bacteria varied and low levels of yeasts and moulds were found. The total number of aerobic bacteria varied from 10×10^1 to 7.5×10^4 cfu/g. *Salmonella* spp. was not detected in any samples. Anaerobic bacteria formed spores in 14.3 to 36.4% of samples, depending on honey type. The number of yeasts and moulds was low and only occasionally exceeded 10×10^2 cfu/g (Rozanska, 2011). Bacteria, such as coliforms, enterococci, bacilli, as well as fungi belonging to the genera *Penicillium*, *Cladosporium*, and *Alteraria* have been identified in Slovak honey (Kacániová et al., 2009). In a study in Turkey on 50 samples collected from retail shops throughout the country, 43 samples contained aerobic mesophilic bacteria at concentrations of 1×10^1 to 9.6×10^6 cfu/g, yeasts were determined in 26 samples and ranged from 1×10^2 to 1.2×10^3 cfu/g, and mould in 46 samples with a concentration of 7.4×10^3 to 1.4×10^5 cfu/g, while *C. botulinum* and *C. perfrigens* were not detected. *B. cereus* with a concentration of 1×10^1 to 1.2×10^2 cfu/g was found in four samples. The authors concluded that during production, storage and sale, honey samples can be contaminated with some microorganisms from various sources, which poses a significant public health risk (Erkan et.al, 2015).

In the present study, the average water content in samples was 17.81%. However, sample no. 19 declared as mountain meadow and sample no. 28 declared as chestnut honey, had moisture contents of 21.10% and 22.20%, respectively, and they do not meet the provisions of the cited Ordinance, due to the increased moisture content. Due to the high sugar content, honey is very hygroscopic and can absorb a certain amount of water in contact with moist air, which can significantly affect the physical properties (crystallization, viscosity and specific gravity), consequently creating problems in processing and storage processes. The moisture content in honey, above all, depends on the maturity of the honey, but also on the season and climatic conditions, as well as storage conditions. The high

moisture content in honey contributes to faster fermentation of honey, its spoilage and loss of organoleptic properties.

The proportion of water in honey is also a very important indicator of the resistance of honey to fermentation and crystallization. The average electrical conductivity was 0.76 mS/cm. Sample no. 31 (meadow honey) did not meet the provisions of the ordinance, as it exceeded the value for electrical conductivity than prescribed by the provisions (0.873 mS/cm). The electrical conductivity of honey is a property that depends primarily on the quantity of mineral salts, organic acids and proteins present. It is also one of the more useful quality parameters in the classification of unifloral honey species (Bogdanov et al., 2008), as the electrical conductivity increases with increasing mineral and acid content in honey (Naila et al., 2018). According to national and international regulations, the electrical conductivity of acacia honey should not exceed 0.8 mS/cm. Chestnut honey, on the other hand, should have an electrical conductivity of at least 0.8 mS/cm. The acidity of honey contributes to its taste and stability, i.e., resistance to microbiological spoilage. In addition to pH value and total acidity, free acidity (free acids) is a useful parameter for distinguishing unifloral honey species (Bogdanov et al., 2008). Free acidity, according to national and international regulations, should not exceed 50 mEq acid per 1000 g honey. Free acids are primarily organic acids such as formic, citric, oxalic, malic and others. The most common is gluconic acid, which is formed from glucose in honey. The higher acid content generally means that the honey has fermented for some time, which has certainly resulted in the conversion of alcohol as a result of fermentation into an organic acid. In the tested samples, the average content of free acids was 30 milieqv ac./1000 g, and sample no. 10 declared as mixed honey 10 (52.28 milieqv ac./1000 g) did not meet the provisions of the regulations on honey quality. The average sucrose content in tested samples was 1.57%. In one sample of flower and one sample of meadow honey, it exceeded 5 g/100 g and therefore this sample is considered unsatisfactory. The maximum amount of sucrose, according to national and international regulations, for most honeys is 5 g/100 g of honey. The exceptions are certain types of honey, including acacia honey with a maximum permitted amount of sucrose of 10 g/100 g of honey.

Considering the quality of the analysed honey samples, 5 of the 33 analysed samples did not meet the requirements for one or more quality parameters. Rončević et al. (2018) reported on the physico-chemical properties of 171 honey samples from Dalmatia in southern Croatia collected from the event Honey Evaluation ("International Beekeeping Fair and Evaluation of Dalmatian Honey") during 2014 and 2015. The determined average share of water for flower honey samples in 2014 was 17.5%, for sage honey 16.2%, for honeydew 16.4%, and in 2015. 16.9% for flower honey, 15.6% for sage honey and 16.0% for honeysuckle honey. The values for electrical conductivity of these samples in 2014 were 0.57 mS/cm for flower honey, 0.27 mS/cm for sage and 1.27 mS/cm for honeysuckle and 0.49 mS/cm, 0.27 mS/cm and 1.01 mS/cm respectively in 2015. The research on the quality of honey conducted in B&H can also be compared with our results.

The physico-chemical analyses of 78 honey samples were made from the entire territory of B&H (2016–2017). The results of average water content were: flower honey 16.86%, sage honey 17.49%, linden honey 16.71%, chestnut honey 16.63% and honeydew 15.54%. Regarding the mean value for electrical

conductivity in B&H, it was 0.31 mS/cm for flower honey, 0.17 mS/cm for sage, 0.16 mS/cm for chestnut honey, and 0.48 mS/cm and 0.27 for honeysuckle mS/cm (Ciric et al., 2018). According to the results obtained in this study, it can be concluded that the values of electrical conductivity of honey samples are similar (Tables 5 and 6).

Conclusion

Among the different types of honey, a limited diversity and small number of microorganisms can be found, indicating a very low level of microbiological contamination. Although honey is known to have antimicrobial and inhibitory potential against pathogenic bacteria, spore-forming bacteria, aerobic mesophilic bacteria, moulds and yeasts, some microorganisms have the ability to survive in honey, which affects the product stability and quality. The number of these microorganisms depends on the flower source and on other factors, such as geographical, climatological, botanical and the origin of honey, although great variability can be observed among the same type of honey. Special attention must be paid to the manipulation of honey during production and storage, and it is necessary to apply good hygiene practices with the application of preventive procedures of self-control of the production process to reduce the public health risk for the general population. Of all the analysed honey samples in this study, inappropriate physico-chemical parameters (reducing sugars, sucrose and total sugars, electrical conductivity, free acid content and moisture content) cannot be correlated with incorrect microbiological analysis results. Considering the obtained test results, it can be concluded that most of the tested samples meet the quality regulations and the microbiological criteria for honey.

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Mikrobiološka i fizikalno-kemijska kvaliteta meda u Bosni i Hercegovini

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Cilj je ovoga rada bio istražiti mikrobiološku i fizikalno-kemijsku kvalitetu uzoraka meda, uzorkovanih za potrebe sajma: "Dani meda u FBiH 2020" u 2020. godini u kojoj su istražena ukupno 33 uzorka različitih vrsta meda s različitog zemljopisnog područja Bosne i Hercegovina (BiH). Cilj je ovog istraživanja bio procijeniti odnos fizikalno-kemijskih osobina i mikrobioloških svojstva ispitivanog meda u svrhu procjene njegove kvalitete. Dostavljeni uzorci su potjecali iz obje administrativno upravne cjeline BiH i to iz kontinentalnog, odnosno submediteranskog i mediteranskog dijela obuhvativši više od 60 % područja države. Od ukupnog broja analiziranih uzoraka (n=33) njih 9 ili 29,7 % nije zadovoljilo zahtjeve fizikalno-kemijskih i mikrobioloških parametara ispitivanja. Zahtjeve jednog ili više parametara kvalitete prema nacionalnom Pravilniku o metodama za kontrolu meda i drugih pčelinjih proizvoda BiH (Anonymous, 2009.) nije zadovoljilo 5 uzoraka (16,5 %). Mikrobiološku kvalitetu zbog prekoračenja dopuštanog broja kvasaca i plijesni. nisu zadovoljila 4 ili 13,2 % analiziranih uzoraka. Kod 5 ili 16,5 % ispitanih uzoraka meda zabilježena je prisutnost pretraženih mikrooganizama u granicama dopuštenih odstupanja, dok *Enterobacteriaceae* i sulfitoreducirajuće klostridije nisu izolirane i bile su ispod limita detekcije. Uzorci meda kod kojih su utvrđeni rezultati bili nezadovoljavajući za fizikalno-kemijske parametre i mikrobiološke parametre ne mogu se dovesti u međusobni odnos.

Ključne riječi: *med, mikrobiološka analiza, fizikalno-kemijska analiza, kvaliteta meda, BiH*