

Characterization of Honey Produced in Bosnia and Herzegovina by Physicochemical and Microbiological Assessment with Microorganism Identification

Azra Bačić¹, Azra Bašić-Halilović¹, Nedžad Prazina², Lejla Skrobo¹, Minela Hukić³

¹Institute for Biomedical Diagnostics and Research, GENOM, Travnik, Bosnia and Herzegovina

²Faculty of Educational Sciences, University of Sarajevo, Bosnia and Herzegovina

³Faculty of Pharmacy and Health, University of Travnik, Bosnia and Herzegovina

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Abstract

This study assessed the quality of 33 honey samples from various regions in Bosnia and Herzegovina by analyzing their physicochemical and microbiological properties. Key findings showed that nearly one-third of samples did not meet national or EU standards in terms of moisture content, pH, acidity, ash, electrical conductivity, and hydroxymethylfurfural (HMF). Although *Salmonella* spp. and *Enterobacteriaceae* were absent from all samples, microbial evaluation revealed that 42% of the honeys exceeded allowable mold counts, and two showed elevated levels of sulphite-reducing clostridia. Molds such as *Cladosporium* spp., *Penicillium* spp., *Mucor* spp., and *Alternaria* spp. were identified. The findings emphasize the importance of maintaining proper hygiene and storage protocols for honey products. Ensuring compliance with food safety regulations is crucial for safeguarding the nutritional and therapeutic value of locally produced honey.

Keywords: Honey characterization, physicochemical analysis, microbiological analysis, molds, Bosnia and Herzegovina.

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*Corresponding Author

Azra Bačić

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INTRODUCTION

Honey is a nutrient-dense natural product synthesized by the honeybee *Apis mellifera* through the collection and enzymatic transformation of floral and tree nectar [1]. This thick, viscous substance is primarily composed of carbohydrates (80–85%), particularly the monosaccharides glucose and fructose, along with a smaller proportion of water (15–20%) [2]. Minor constituents such as minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, and phenolic compounds account for less than 1.5% of its composition [3]. This complex biochemical structure contributes to honey's antimicrobial properties [4] and supports its antioxidant and antiproliferative activity [5]. Low pH, elevated sugar levels, and minimal water activity in honey collectively form a hostile environment that inhibits microbial proliferation, as supported by prior studies [6]. Nevertheless, honey

can still harbor a variety of microorganisms due to contamination before and after harvesting. Primary contamination sources include pollen, nectar, air, dust, and the digestive tract of honeybees, while secondary sources involve improper handling practices and unsanitary equipment or storage environments [7]. As noted by Kačániová et al., aerobic mesophilic bacteria form part of the natural bee gut microbiota, with species such as *Bacillus*, *Clostridium*, *Saccharomyces*, *Penicillium*, *Mucor*, *Schizosaccharomyces*, and *Torula* being commonly present [8]. Among these, sulfite-reducing *Clostridium* species are considered indicators of environmental contamination [9] and their spores pose a particular health risk due to their association with infant botulism [10]. Additionally, the presence of free amino acids, sugars, and minerals, especially under suboptimal storage conditions, makes honey susceptible to yeast and mold proliferation [11]. The chemical profile and physicochemical attributes of honey vary significantly based on factors such as floral origin, geographic region, climate, and bee species [12]. According to European regulations, honey quality is determined by parameters including moisture content,

electrical conductivity, ash content, levels of reducing and non-reducing sugars, free acidity, diastase activity, and hydroxymethylfurfural (HMF) concentration. Among these, water content is particularly critical, serving as a key indicator of honey's maturity and long-term stability [13]. This study aimed to evaluate and characterize various honey types produced in Bosnia and Herzegovina by analyzing their physicochemical and microbiological properties, with a focus on mold identification.

MATERIALS

Study Area

This study analyzed 33 natural honey samples collected from various regions across Bosnia and Herzegovina, a country situated in the western Balkans, covering an area of 51,209.2 km². The geographical diversity of Bosnia and Herzegovina is reflected in its varied climate, vegetation, and topography. The climate ranges from temperate continental conditions in the north, to alpine climates in the mountainous central areas, and Mediterranean influences in the southern Herzegovina region.

Sampling

Honey samples were collected from professional beekeepers during the final production phase, spanning June to September 2022 (Figure 1). A total of six honey types were sampled: meadow ($n = 17$), polyfloral ($n = 4$), acacia (*Robinia pseudoacacia*) ($n = 4$), meadow-forest ($n = 3$), sage (*Salvia officinalis* L.) ($n = 2$), and chestnut (*Aesculus hippocastanum*) ($n = 3$). Crude honey was obtained by centrifugation, after which the uppermost layer was removed. Approximately 200 g of each sample was aseptically transferred into sterile plastic containers. Samples were labeled and stored at ambient temperatures (18–27°C) until further analysis. All laboratory analyses were conducted at the Institute for Biomedical Diagnostics and Research Genom Travnik, an ISO 17025-accredited facility.

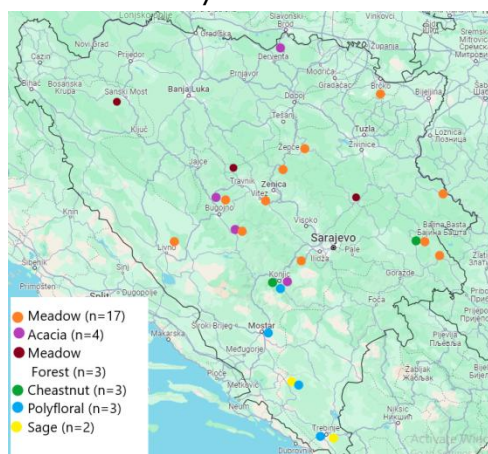


Figure 1. Geographical location of honey sampling sites

METHODS

The chemical parameters of the honey samples were determined following the procedures described by Bogdanov [14]. Water content was measured by drying 5 g of honey in an oven at 103°C for 2 hours, with subsequent weighing. For pH determination, honey was diluted in 10% distilled water and measured using a pH meter (Eutech, pH700). Ash content was assessed by incinerating 3 g of honey in a muffle furnace at 600°C for 2 hours. Sugar content was quantified using the Luff-Schoorl method, which relies on the reduction of Cu²⁺ ions to Cu⁺ ions by reducing sugars under specific conditions. Electrical conductivity was measured with a conductometer (Eutech, CON 700) at 20°C, using a 20% aqueous honey solution prepared based on the dry matter content. Diastase activity was determined using the Schade method, which evaluates the enzymatic degradation of the blue starch–triiodide complex under standard conditions; the decrease in blue coloration was monitored spectrophotometrically at set time intervals. Hydroxymethylfurfural (HMF) content was determined using a spectrophotometric method. Two milliliters of each diluted honey sample were mixed with 5 mL of p-toluidine in test tubes. For the reference solution, 1 mL of distilled water was added, while the test sample received 1 mL of barbituric acid. After thorough mixing, the absorbance of the sample solution was measured against the reference at 550 nm using a Shimadzu UV-1800 spectrophotometer. Free acidity was assessed by titration. The honey solution was titrated with 0.05 N sodium hydroxide (NaOH) until a pH of 8.50 was reached. Results were expressed as milliequivalents of acid per kilogram of honey. Microbiological analyses included total viable count, *Enterobacteriaceae*, sulfite-reducing anaerobic bacteria, yeasts, molds, and *Salmonella* spp. For each sample, 10 g of honey was homogenized in 90 mL of buffered peptone water, and serial decimal dilutions were prepared.

Total mesophilic aerobic count was performed in accordance with BAS EN ISO 4833-1:2014 [15]. Briefly, 1 mL of the 10⁻¹ and 10⁻² dilutions was aseptically transferred into sterile Petri dishes, followed by the addition of 10–15 mL of Plate Count Agar (PCA). After solidification, plates were inverted and incubated at 30°C for 72 hours.

Enumeration of *Enterobacteriaceae* followed BAS EN ISO 21528-2:2018 [16]. One millilitre of each dilution (10⁻¹ and 10⁻²) was inoculated into sterile plates. Melted Violet Red Bile Glucose Agar (VRBG), cooled to 45°C, was added (15 mL per plate), followed by a 5

mL overlay after the first layer solidified. Plates were incubated at 37°C for 24–48 hours. Sulfite-reducing anaerobic bacteria were enumerated using the ISO 15213:2008 method [17]. One milliliter of each dilution (10^{-1} and 10^{-2}) was placed into sterile tubes with 20 mL of molten Iron Sulfite Agar (ISA) at 45°C. After mixing and solidification, tubes were incubated anaerobically at 37°C for 24–48 hours.

Yeasts and molds were quantified following BAS EN ISO 21527-2:2009 [18]. A volume of 0.1 mL from the stock solution and its 10^{-1} and 10^{-2} dilutions was spread onto Dichloran Glycerol 18% (DGI8) agar and incubated at 25°C for 5 days.

Mold identification to the genus level was performed using Czapek Yeast Extract Agar and Malt Extract Agar for colony purification. Identification was based on macroscopic and microscopic examination of colony morphology and spore structures, as described by Pitt and Hocking [19].

Detection of *Salmonella* spp. was carried out using 25 g of honey mixed with 225 mL of buffered peptone water and incubated at 37°C for 18 hours for pre-enrichment. Selective enrichment was performed using Rappaport-Vassiliadis (RVS) broth incubated at 41.5°C and Muller-Kauffmann Tetrathionate-Novobiocin (MKTn) broth incubated at 37°C, both for 24 hours. Isolation was conducted on Xylose Lysine Deoxycholate (XLD) agar and Salmonella–Shigella (SS) agar, incubated at 37°C for 24 hours [20].

RESULTS

The physicochemical parameters of the honey samples are summarized in Table I. Of the 33 samples analyzed, 10 (30.30%) did not meet the required physicochemical quality standards [21] for one or more parameters (Table I).

Table I. Physicochemical analysis of honey samples

S.No	Honey type	Water content, %	pH	Ash, %	Sugar content, %	Electrical conductivity, mS/cm	Diastase activity, mEq/kg	HMF, mg/kg	Acidity, mEq/kg
1	Meadow 1	15.58	4.6	0.21	71.7	0.62	20.4	9.4	37.4
2	Meadow 2	16.12	4.7	0.17	69.8	0.72	10.1	25.7	35.6
3	Meadow 3	15.89	4.8	0.11	74.5	0.39	41.7	2.2	33.8
4	Meadow 4	17.14	4.8	0.19	68.7	0.54	35.4	5.1	31.6
5	Meadow 5	15.08	4.9	0.23	74.3	0.61	36.1	3.3	32.6
6	Acacia 1	18.11	4.5	0.11	80.1	0.75	29.9	8.0	35.1
7	Meadow 6	17.89	3.9	0.16	79.3	0.32	12.8	25.5	30.3
8	Meadow 7	19.22	4.5	0.25	64.5	0.20	40.1	1.2	31.2
9	Meadow 8	17.74	4.6	0.22	74.8	0.38	20.7	13.2	29.9
10	Meadow 9	16.25	4.5	0.28	72.1	0.45	38.5	4.1	32.9
11	Meadow 10	16.78	4.8	0.14	78.3	1.57	19.7	10.9	22.5
12	Meadow 11	15.89	4.4	0.17	75.9	0.89	21.4	9.9	35.8
13	Meadow 12	17.12	4.6	0.19	63.8	0.74	20.9	9.3	34.9
14	Sage 1	16.42	4.5	0.21	67.2	2.14	37.1	6.4	41.6
15	Meadow Forest 1	18.21	4.5	0.14	72.3	0.84	24.6	12.5	33.9
16	Acacia 2	17.22	3.8	0.14	78.6	0.69	22.1	12.2	33.7
17	Chestnut 1	15.24	4.6	0.11	67.8	0.87	41.2	5.0	19.9
18	Meadow 13	16.78	4.2	0.25	78.4	2.09	28.6	13.1	20.4
19	Meadow	17.12	3.8	0.17	69.5	0.25	23.8	14.4	34.2

	14								
20	Meadow 15	15.19	4.5	0.24	75.1	0.61	47.2	5.5	28.8
21	Meadow 16	16.78	3.7	0.17	72.8	0.53	9.5	29.4	40.4
22	Polyfloral 1	18.09	4.3	0.19	76.2	0.34	30.5	12.1	34.9
23	Sage 2	17.24	4.7	0.17	70.5	0.82	22.5	9.9	35.2
24	Polyfloral 2	17.28	4.0	0.28	78.3	0.46	28.7	7.9	30.2
25	Meadow Forest 2	19.08	4.0	0.21	70.5	0.84	6.1	213.1	26.1
26	Polyfloral 3	16.25	4.5	0.33	71.8	0.36	10.2	35.1	30.7
27	Chestnut 2	15.74	4.5	0.15	70.1	0.96	27.4	15.6	55.1
28	Acacia 3	16.87	3.8	0.12	81.5	0.61	25.6	11.1	67.4
29	Polyfloral 4	17.56	3.8	0.25	67.4	0.29	9.7	20.7	30.7
30	Meadow Forest 3	16.17	3.9	0.18	69.1	0.41	9.5	19.5	35.9
31	Meadow 17	20.01	4.8	0.24	71.8	0.59	17.5	12.2	26.1
32	Acacia 4	17.11	3.7	0.09	74.9	1.17	16.7	15.7	80.7
33	Chestnut 3	16.25	4.6	0.07	62.8	1.12	11.4	11.2	20.3

Mean values in all analyzed types of honey were 16.95 %; 4.36; 0.19%; 72.56%, 0.73 mS/cm; 24.17 mEq/kg; 18.50 mg/kg and 34.84 mEq/kg for water content, pH, ash, sugar, electrical conductivity, diastase activity, HMF and free acidity, respectively (Table 2.).

Table 2. Average results of parameters according to honey type

Honey type	Physicochemical properties							
	Water content,%	pH	Ash,%	Sugar content,%	Electrical conductivity, mS/cm	Diastase activity, mEq/kg	HMF, mg/kg	Acidity, mEq/kg
Meadow mean \pm SD min max	16.9 \pm 0.7	4.5 \pm 0.3	0.2 \pm 0.2	72.7 \pm 0.6	0.7 \pm 0.2	26.1 \pm 0.8	11.4 \pm 0.5	31.7 \pm 0.8
	15.1	3.7	0.1	63.8	0.2	9.5	1.2	20.4
	20.0	4.9	0.3	79.3	2.1	47.2	29.4	40.4
Polyfloral mean \pm SD min max	17.6 \pm 0.7	4.3 \pm 0.3	0.26 \pm 0.2	73.4 \pm 0.6	0.39 \pm 0.2	19.8 \pm 0.8	18.4 \pm 0.5	31.9 \pm 0.8
	17.3	4.0	0.19	67.4	0.34	9.7	7.9	30.2
	18.1	4.5	0.33	78.3	0.46	30.5	35.1	34.9
Meadow forest mean \pm SD Min Max	17.8 \pm 0.7	4.1 \pm 0.3	0.18 \pm 0.2	70.6 \pm 0.6	0.70 \pm 0.2	13.4 \pm 0.8	79.3 \pm 0.5	32.0 \pm 0.8
	16.2	3.9	0.14	69.1	0.41	6.1	12.2	26.1
	19.1	4.5	0.21	72.3	0.84	24.6	213.1	35.9
Acacia mean \pm SD Min Max	17.4 \pm 0.7	4.0 \pm 0.3	0.1 \pm 0.2	80.1 \pm 0.6	0.7 \pm 0.2	25.9 \pm 0.8	10.4 \pm 0.5	45.4 \pm 0.8
	16.9	3.8	0.1	78.6	0.6	22.1	8.0	33.7
	18.1	4.5	0.1	81.5	0.8	29.9	12.2	67.4
Sage mean \pm SD Min Max	16.8 \pm 0.7	4.6 \pm 0.3	0.19	68.9 \pm 0.6	1.48 \pm 0.2	29.8 \pm 0.8	8.2 \pm 0.5	38.4 \pm 0.8
	16.4	4.5	0.17	67.2	0.82	22.5	6.4	35.2
	17.2	4.7	0.21	70.5	2.14	37.1	9.9	41.6
Chestnut mean \pm SD Min max	15.7 \pm 0.7	4.6 \pm 0.3	0.1 \pm 0.2	66.9 \pm 0.6	1.0 \pm 0.2	26.7 \pm 0.8	10.6 \pm 0.5	31.8 \pm 0.8
	15.2	4.5	0.1	62.8	0.9	11.4	5.0	19.9
	16.3	4.6	0.2	70.1	1.1	41.2	15.6	55.1

SD: standard deviation; Min: minimum value; Max: maximum value

The results of the microbiological analysis are presented in Table 3. *Enterobacteriaceae* and *Salmonella* spp. were not detected in any of the 33 honey samples. However, 14 samples (42.42%) did not meet the microbiological quality standards due to yeast and mold counts exceeding the permissible limit of 10^2 CFU/g, in accordance with the Guidelines on Microbiological Criteria for Food in Bosnia and Herzegovina [22]. Additionally, two samples (6.06%) exceeded the allowable limit of 10 CFU/g for sulfite-reducing clostridia. Aerobic mesophilic bacteria were detected in 12 samples, but their counts remained within the acceptable limits.

Table 3. Microbiological analysis of honey samples

S.No	Honey type	Mesophilic aerobic bacteria/g	Enterobacteriaceae/g	Sulfite-Reducing Anaerobic Bacteria/g	Yeasts and Molds/g	Salmonella spp./g
1	Meadow 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
2	Meadow 2	2×10 cfu/g	<10 cfu/g	<10 cfu/g	3×10^2 cfu/g	absence
3	Meadow 3	4×10 cfu/g	<10 cfu/g	<10 cfu/g	3×10^2 cfu/g	absence
4	Meadow 4	2×10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
5	Meadow 5	1.1×10^2 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
6	Acacia 1	5.1×10^3 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
7	Meadow 6	<10 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
8	Meadow 7	8.3×10^3 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
9	Meadow 8	4×10 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
10	Meadow 9	<10 cfu/g	<10 cfu/g	10 cfu/g	2×10^2 cfu/g	absence
11	Meadow 10	<10 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
12	Meadow 11	10 cfu/g	<10 cfu/g	<10 cfu/g	7×10^2 cfu/g	absence
13	Meadow 12	<10 cfu/g	<10 cfu/g	<10 cfu/g	3×10^2 cfu/g	absence
14	Sage 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
15	Meadow Forest 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
16	Acacia 2	10 cfu/g	<10 cfu/g	<10 cfu/g	1×10^2 cfu/g	absence
17	Chestnut 1	4×10 cfu/g	<10 cfu/g	<10 cfu/g	5×10^2 cfu/g	absence
18	Meadow 13	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
19	Meadow 14	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
20	Meadow 15	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
21	Meadow 16	10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
22	Polyfloral 1	<10 cfu/g	<10 cfu/g	<10 cfu/g	1×10^2 cfu/g	absence
23	Sage 2	1.4×10^2 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
24	Polyfloral 2	10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
25	Meadow Forest 2	7.1×10^2 cfu/g	<10 cfu/g	5×10 cfu/g	<10 cfu/g	absence
26	Polyfloral 3	5×10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
27	Chestnut 2	7×10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
28	Acacia 3	10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
29	Polyfloral 4	<10 cfu/g	<10 cfu/g	<10 cfu/g	2×10^2 cfu/g	absence
30	Meadow Forest 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
31	Meadow 17	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
32	Acacia 4	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence
33	Chestnut 3	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	absence

Table 4. Molds identification results

Mold genus	The number of mold isolates in different types of honey						
	Meadow	Polyfloral	Meadow forest	Acacia	Sage	Chestnut	Total
<i>Cladosporium</i> spp.	5	1	-	-	-	-	6
<i>Penicillium</i> spp.	4	-	-	1	1	-	6
<i>Mucor</i> spp.	-	-	-	1	-	-	1
<i>Alternaria</i> spp.	-	-	-	-	-	1	1
Total	9	1	-	2	1	1	14

We identified following molds: *Cladosporium* spp., *Penicillium* spp., *Mucor* spp., and *Alternaria* spp. (Table 4.)

DISCUSSION

In this study, the average water content in honey samples was 16.95%. One meadow honey sample exceeded the maximum permissible limit, with a moisture content of 20.01%. Due to its high sugar content, honey is highly hygroscopic and can absorb moisture from humid air, increasing the risk of fermentation and spoilage by mold. Although Snowden and Cliver [7] identified moisture levels above 20% as a key factor in mold development, our results showed no correlation between moisture or acidity and yeast and mold counts. The average pH and ash content were 4.36 and 0.19%, respectively, consistent with values reported by Akyuz et al. [23]. The sugar profile of honey is dominated by monosaccharides like fructose and glucose, along with disaccharides such as sucrose and maltose. Together, glucose and fructose constitute the majority of honey's carbohydrate content, shaping its physical properties-such as crystallization behavior, viscosity, and its ability to retain moisture [24]. The average total sugar content in our samples was 72.56%, with all samples exceeding the 60% minimum threshold. The average electrical conductivity was 0.73 mS/cm. Five samples-three meadow, one sage, and one acacia-exceeded the recommended maximum of 0.8 mS/cm for certain honey types. Honey's electrical conductivity reflects its concentrations of minerals, organic acids, and proteins, and varies with botanical origin; higher values suggest a greater concentration of these components [25]. According to both national and EU regulations, acacia honey should not exceed 0.8 mS/cm, while chestnut honey should be at least 0.8 mS/cm. Diastase activity is a key indicator of honey heating during processing and storage, as it decreases with heat exposure. One sample exceeded the diastase activity threshold of 8 mEq/kg and also exhibited elevated HMF levels, suggesting possible overheating or adulteration. HMF content in the analyzed samples ranged from 1.2 to 213.1 mg/kg, with one sample exceeding the regulatory limit of 40 mg/kg. HMF is a heat-induced degradation product of sugars in acidic conditions and is often used as a marker of honey freshness or authenticity [26]. High HMF levels may indicate counterfeit honey and pose a potential public health risk, highlighting the importance of routine physicochemical monitoring [27]. The free acidity of honey, primarily derived from formic, citric, oxalic, and malic acids, should not exceed 50 mEq/kg. Elevated acidity can indicate

fermentation. The average free acidity across samples was 34.84 mEq/kg; however, three samples exceeded the limit. These findings align with those of Landeka et al. for honey produced in Bosnia and Herzegovina [28]. No correlation was observed between unsatisfactory physicochemical and microbiological parameters in the same samples. *Enterobacteriaceae* and *Salmonella* spp. were absent in all samples. Total mesophilic aerobic bacterial counts ranged from 10 to 8.3×10^3 CFU/g, within the permissible limit of $<10^4$ CFU/g under the B&H Codex Alimentarius [22]. These results are similar to those reported by Landeka et al. for Bosnian floral honey (maximum 9.3×10^3 CFU/g) [28], though higher than counts reported in Algeria [29] and Romania [30]. Sulfite-reducing anaerobic bacteria were found in two samples (6.06%) with counts of 10 and 50 CFU/g, indicating contamination. In contrast, Landeka et al. [28] reported no clostridia in Bosnian honey, while Nevas et al. [31] documented high contamination rates in Finland and other regions. Fungal counts (molds) ranged from 1×10^2 to 7×10^2 CFU/g in 14 samples (42.42%) exceeding the recommended 10^2 CFU/g limit per the B&H Guidelines. These findings are similar to those of Ananias et al. [32], who reported inadequate quality in 45.7% of Brazilian samples. In comparison, studies from Portugal and Romania found much lower mold levels (<40 CFU/g) [30, 33]. Identified mold genera included *Cladosporium*, *Penicillium*, *Mucor* and *Alternaria*, commonly found in honey, as also reported in Croatian honey by Kiš et al. [11]. Although these fungi typically do not proliferate in honey, high counts are indicative of environmental or equipment-related contamination and reflect poor hygiene during processing [7]. This emphasizes the need for quality control programs targeting hygiene practices in post-harvest processing [32]. High mold counts contribute to increased acidity by promoting fermentation, where sugars are converted to alcohol and subsequently to acetic acid [34]. Osmophilic yeasts, capable of growing in low-pH environments, are responsible for honey spoilage and shortened shelf life [35].

CONCLUSION

A total of 69.70% of the honey samples complied with the physicochemical and microbiological quality criteria established by national regulations in Bosnia and Herzegovina, while only 57.58% met the standards outlined by the European Union directives. Considerable variation was observed among samples in terms of physicochemical properties. Parameters such as moisture content, pH, free acidity, ash, electrical conductivity, and hydroxymethylfurfural (HMF) levels

exceeded permissible limits in 30.30% of the samples. Microbiological analysis also revealed significant differences in microbial profiles. Specifically, sulfite-reducing clostridia levels surpassed acceptable thresholds in 2 samples (6.06%), while mold contamination exceeded regulatory limits in 14 samples (42.42%), representing a substantial proportion of unsatisfactory microbiological quality. This study aimed to characterize and classify various types of honey produced in Bosnia and Herzegovina by evaluating their physicochemical and microbiological profiles. Ensuring and enhancing the quality of locally produced honey is essential, particularly considering its widespread use for both nutritional and therapeutic purposes, owing to its well-documented antibacterial properties.

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Conflict of Interest

Authors declare no conflict of interest

Informed Consent

Not applicable.

Ethical Statement

Not applicable.

Author Contributions

Azra Bačić - conceptualization, methodology, data analysis, writing; Azra Bašić-Halilović - conceptualization, methodology, data analysis, writing; Nedžad Prazina- data analysis, writing; Lejla Skrobo – methodology; Minela Hukić - methodology;

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