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# Health Safety and Sensory Properties of Honey from Two Different Areas in Tuzla Canton

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**ABSTRACT**

**People's interest in the safe use of food has existed since ancient times, so that even today there is a global demand for the production of food that can be used without risk to health. Contaminants, as well as the type and location of grazing can affect the physico-chemical and sensory properties of honey. The aim of the study was to examine the microbiological validity and sensory properties of honey from two different areas in Tuzla Canton. The research included three samples of forest honey from the Kladanj area and three samples of flower honey from the Lukavac area. In addition to microbiological analyzes and sensory properties, the concentration of HMF in honey was determined as an indicator of heating and inappropriate storage of honey. All samples were microbiologically correct.**

**The sample of flower honey L1, which did not meet the criteria prescribed by the Regulation on Honey and Other Bee Products, was not sensory evaluated. L1 sample was not sensory evaluated due to an increased HMF content of 105.38 mg/kg.**

**Based on sensory analysis, the samples of flower honey from Lukavac were better evaluated than forest honey from Kladanj. Statistical analysis showed that there is a significant difference between the mean values for the sensory properties of purity and color.**

***Keywords-*** honey, healt safety, HMF, sensory analysis.

## I. INTRODUCTION

Honey is a completely natural product that can be used directly from the hive in its natural form, i.e., in that state in which the bees produced it. Honey is a natural complex mixture of very different chemical ingredients, primarily different sugars, mainly fructose and glucose, water and other substances that reach the honey during its production, produced by the honey bee (*Apis mellifera*) and, regardless of industry progress, cannot be replaced by a production process. The presence of specific substances (such as phenols, flavonoids, aromatic acids), acidity, osmolality, and enzymatic formation of hydrogen peroxide in honey inhibit the growth and multiplication of pathogenic bacteria and food spoilage agents [1] (Kiš et al., 2019). There are two sources of honey contamination. The primary source is the bee's digestive system, dust, soil, pollen, nectar and air. Contaminants that occur during the technological process of honey production and storage are secondary sources of contamination. Microorganisms of concern in honey are some fungi and spore-forming bacteria, such as *Bacillus cereus* and *Clostridium* spp. which, under certain conditions, could cause disease affecting humans [2] (Snowdon et al., 1996). Fungi in honey produce mycotoxins, which are very toxic to humans even in small concentrations. Microbiological criteria on permitted species and number of microorganisms, bacterial toxins and histamines in food dangerous to health and microbiological criteria for hygiene of production processes, determination and assessment methods are prescribed by the Regulation on Microbiological Criteria for Food [3] ("Official Gazette of Bosnia and Herzegovina, No. 11/13). The maximum allowable concentration (MAC) for aerobic mesophilic bacteria is $10^3$ CFU/g, for *Enterobacteriaceae* is $10^2$ CFU/g, sulfite reducing clostridia 10 CFU/g, yeast $10^2$ CFU/g and for molds the maximum allowable concentration is $10^3$ CFU/g. The hydroxymethylfurfural content is one of the criteria for honey quality [4,5] (Alwarez et al., 2010; Lazarević et al., 2012;) and can serve as a freshness indicator [6] (Naila et al., 2018), heating and storage of honey [7] (Batinić and Palinić, 2014) because it is usually absent in fresh honey or is present in very small concentrations [8] (Shapla et al., 2018). HMF is present in small amounts and in natural honey immediately after honey extraction. The proportion of HMF primarily depends on the type of honey, pH value, acid content, moisture and light exposure. Therefore, good beekeeping practice, but also the conditions of distribution and storage of honey, are crucial for the final low content of HMF.

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Today, the proportion of HMF is primarily used as an indicator of freshness and heating of honey, but extremely high values above 100 mg/kg can still be a good indicator of possible adulteration of honey. According to the Regulation on Honey and Other Bee Products [9] ("Official Gazette of Bosnia and Herzegovina", No. 37/09), the content of HMF for honey in general must not exceed 40 mg/kg.

Sensory analysis is used to confirm honey quality [10] (Marcazzan et al., 2017) to complete the physicochemical analysis of honey and pollen [11] (Moumeh et al., 2020). The composition and sensory properties of honey depend primarily on the nectar botanical origin, climatic conditions and soil properties on which the plant is distributed, the breed of bees and the abilities of the beekeeper himself.

## II. EXPERIMENTAL PART

### *2.1. Materials*

Honey samples come from private beekeeping production from two different locations:

- 3 samples of forest honey from the area of the Kladanj municipality (K1, K2, K3),
- 3 samples of flower honey from the area of Lukavac municipality (L1, L2, L3).

Bee grazing in all 3 samples from the area of the Kladanj municipality is performed on meadows and nearby forests in the vicinity of which there are no industrial plants and other forms of ecosystem pollution. Unlike the samples from the Kladanj municipality, the samples from the area of the Lukavac municipality are in relative proximity to large industrial plants and other potential pollutants of the ecosystem. Bee grazing in samples from the area of Lukavac municipality is performed mainly in meadows and to a lesser extent in forests.

During the analyzes, the honey was stored at 20°C in the dark places.

### *2.2. Methods*

#### 2.2.1. Microbiological quality of honey

Ten grams of each honey sample were homogenized into 90 mL of peptone water solvent. Decimal dilutions of sample were made into the same solvent. Aerobic mesophilic bacteria were counted onto standard plate count agar (PCA) and incubated at 30°C for 48 h [12] (ISO 4833-2:2014). Moulds and yeasts counts followed the protocol of [13] ISO 21527-2:2009. Microbial counts were expressed as colony-forming units per gram of honey (cfu/g).

$$CFU = \frac{number\ of\ colonies}{volume * dilution factor}$$

For sulphite-reducing clostridia counting, aliquots of 10, 5, 1 and 0.1 mL of the initial suspension were added to an empty tube, thermally treated at 80 °C for 5 min and covered with SPS (sulphite–polymixin–sulfadiazine) agar media, tubes were incubated at 37 °C for 5 days [14] (ISO 15213:2003). Fecal coliforms *(Enterobacteriaceae*) were enumerated by the Most Probable Number technique defined in the ISO protocol 21528-2:2017 [15] .

#### *2.2.2. Determining hydroxymethylfurfural content by White*

Determining HMF content is based on determining HMF absorbance at 284 nm. To avoid the influence of other absorbent components at 284 nm, the difference is determined between the absorbance of the pure sample solution and the solution to which the aldehyde group-blocking Na-bisulfite was previously added. The HMF content is calculated after subtraction of absorbance at 336 nm according to the following formula:

$$HMF\ [mg/kg] = (A_{284} - A_{336}) \cdot 149{,}7 \cdot 5\ D/W$$

Where: $A_{284}$ is absorbance at 284 nm;

$A_{336}$ is absorbance at 336 nm;

149,7 is the factor;

D is the dilution factor (if dilution is required);

W is the honey sample mass.

The method by White can be applied to all types of honey.

#### *2.2.3. Sensory analysis*

Sensory analysis of honey samples was conducted by a group of 5 analysts, who have experienced in sensory evaluation of honey. Procedures, condition of preparation and serving of samples were performed according to [16] (Araujo D., *et al*. (2020). Forty grams of each sample was put into a glass vial and covered with a watch glass for sensory analysis. The samples were prepared one hour before tasting to achieve the equilibrium of the headspace and they were served at 20ºC. Four samples, labelled with three-digited random numbers, were served, one at a time, over a session. Mineral water and apple were used to cleanse the palate between samples.

Seven sensory attributes were evaluated, three for appearance (color, purity and clarity), one for odour, one for taste and two for aroma (characteristic of honey type and presence (strength) of aroma). The attributes were evaluated with different points: points from 1 to 3 for odour and purity, points from 1 to 4 for purity and presence (strength) of aroma, points from 1 to 5 for odour and taste and points from 1 to 6 for characteristic of honey type. The minimum sum of points in the overall rating for appearance is 3 points, the maximum is 10 points. The minimum sum of points in the overall rating for aroma is 2 points, the maximum is 10 points [17] (Brčina T., *et al*, (2021.)

#### *2.2.4. Statistical analysis*

Correlation and analysis of variance (ANOVA) was carried out using SPSS software (version 25). Tukey test was used to determinate which samples were

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statistically different by all the sensory properties and acceptance of product (P<0.05).

## III. RESULTS AND DISCUSION

Tables 1 to 3 show the results of microbiological analysis of honey, HMF content (mean value ± SD), sensory analysis of honey samples (mean value ± SD).

**Table1: Microbiological analysis of honey**

| Sample | Sulphite-reducing clostridia (CFU /g) | Moulds and yeasts (CFU /g) | Fecal coliforms (*Enterobacteriacea*) (CFU /g) | Aerobic mesophilic bacteria (CFU g) |
|---|---|---|---|---|
| K1 | 0 | 0 | 0 | 8200 |
| K2 | 0 | 0 | 0 | 2000 |
| K3 | 0 | 0 | 0 | 1300 |
| L1 | 0 | 0 | 0 | 7600 |
| L2 | 0 | 0 | 0 | 2300 |
| L3 | 0 | 0 | 0 | 1900 |

The mean CFU value of aerobic mesophilic bacteria, for all tested samples, was ranged between 1.3 * $10^3$ CFU / g and 8.2 * $10^3$ CFU / g. Such results of microbiological analysis are satisfactory, ie the number of grown colonies was within the allowed limits.

In respect to sanitary quality (fecal coliforms) and safety (sulphite-reducing clostridia), as well as the presence of yeasts and molds, all samples were negative.

**Table 2: HMF in honey**

| Sample | HMF (mg/kg) (± SD) |
|---|---|
| K1 | 0.88 (±0.00) |
| K2 | 1.90 (±0.06) |
| K3 | 22.89 (±0.06) |
| L1 | 105.38 (±3.84) |
| L2 | 17.06 (±0.29) |
| L3 | 28.04 (±0.25) |

HMF values in the samples range from 0.8832-105.3888 mg/kg. Forest honey samples from the Kladanj area had lower HMF concentrations compared to flower honey samples from the Lukavac area.

The lowest HMF content has a sample of forest honey K1 from the area of Kladanj municipality, while the highest HMF value has a sample of flower honey L1 from the Lukavac municipality, which is about a hundred times higher than the K1 sample, and about five times higher than the K3 sample. According to the Regulations on Honey and Other Bee Products [9] ("Official Gazette of Bosnia and Herzegovina", No. 37/09), the maximum permitted concentration of HMF is 40 mg/kg, which means that the values for the K1, K2, K3, L2, L3 samples are in accordance with the parameters prescribed by the Regulations. The flower honey sample L1 with HMF content of 105.3888 mg/kg, does not comply with the criteria prescribed by the Regulations on Honey and Other Bee Products [9] ("Official Gazette of Bosnia and Herzegovina", No. 37/09). HMF is an important parameter for determining the honey purity. High HMF content may be associated with inadequate honey storage conditions. Many authors have observed the connection between HMF content and physicochemical characteristics of honey [18, 19] (Khallil et al., 2010; Fallico et al., 2004), as well as the influence of metal ions on increased HMF content [20] (OumaáAnam, 1995). Compared to the heavy metals content in the same examined samples, which were published in the paper [21] (Seferović et al., 2019), the content of manganese and lead had an impact on the increased HMF content in honey samples from the Kladanj area, but are not statistically significant. In the samples from the Lukavac area there is a positive correlation between the lead and HMF content and it is statistically significant (r=0.999; p<0.05).

L1 sample was not sensory evaluated due to an increased HMF content of 105.3888 mg/kg. This value is almost three times higher than the value defined by the Regulations on Honey and Other Bee Products [9] ("Official Gazette of Bosnia and Herzegovina", No. 37/09), and for the heath protection of the evaluators, this sample was not submitted to them for evaluation.

Based on the results shown in Table 3, the flower honey samples from the Lukavac area were better evaluated compared to forest honey from the Kladanj area. Flower honey received higher marks for purity, clarity, aroma, characteristic type of honey and presence of aroma, while forest honey was better rated for color and taste.

Based on the results, K2 sample is the best and K3 sample the worst evaluated by the evaluators, which is shown by the total number of points.

Analysis of variance (Table 4) showed that there is a statistically significant difference (p> 0.05) between the mean values for the sensory properties of purity and color. Since there is a statistically significant difference (p <0.05), a Tukey test was performed, based on which it was determined in which honey samples the sensory properties of purity and color were better assessed. L3, K1, K2 and L2 samples were best rated for purity. The color of L2, L3, K1 and K2 samples was better evaluated than the color of K3 sample. The visual appearance of honey, such as the color and clarity itself, are a valuable and first indicator when assessing the honey quality and determining potential adulteration. As for the other sensory parameters, there was no statistically significant difference between the samples.

**Table 3: Sensory analysis of honey**

| Sample | Purity (1-3) | Color (1-4) | Clarity (1-3) | Smell (1-5) | Taste (1-5) | Characteristic of honey type (1-6) | Presence (strength) of aroma (1-4) |
|---|---|---|---|---|---|---|---|
| K1 | 3 (±0)[2] | 4 (±0) | 3 (±0)[2] | 4.2 (±0.836) | 4.6 (±0.547) | 5.2 (±0.836) | 3.6 (±0.547) |
| K2 | 3 (±0)[2] | 3.8 (±0.447) | 3 (±0)[2] | 4.6 (±0.544) | 4.8 (±0.557) | 5.2 (±0.836) | 3.8 (±0.447) |
| K3 | 1.8 (± 0.836)[1] | 3.6 (±0.547) | 1.8 (±0.447)[1] | 3.4 (±1.140) | 3.6 (±0.547) | 3.8 (±1.095) | 2.8 (±0.836) |
| L2 | 3 (±0)[2] | 3.6 (±0.547) | 2.8 (±0.447)[2] | 4.0 (±1.000) | 4.2 (±0.836) | 5 (±0.707) | 3.4 (±0.547) |
| L3 | 2.4 (±0.894)[1.2] | 3.6 (±0.894) | 2.8 (±0.447)[2] | 4.2 (±1.303) | 4.4 (±1.341) | 5.2 (±1.788) | 3.6 (±0.894) |

**Table 4: Analysis of variance of data from table 3**

| | | Sum of Squares | df | Mean square | F | p |
|---|---|---|---|---|---|---|
| Purity | Between Groups | 5.760 | 4 | 1.440 | 4.800 | 0.007 |
| | Within Groups | 6.000 | 20 | 0.300 | | |
| | Total | 11.760 | 24 | | | |
| Color | Between Groups | .640 | 4 | 0.160 | .500 | 0.736 |
| | Within Groups | 6.400 | 20 | 0.320 | | |
| | Total | 7.040 | 24 | | | |
| Clarity | Between Groups | 5.040 | 4 | 1.260 | 10.500 | 0.000 |
| | Within Groups | 2.400 | 20 | 0.120 | | |
| | Total | 7.440 | 24 | | | |
| Smell | Between Groups | 3.840 | 4 | 0.960 | .960 | 0.451 |
| | Within Groups | 20.000 | 20 | 1.000 | | |
| | Total | 23.840 | 24 | | | |
| Taste | Between Groups | 4.240 | 4 | 1.060 | 1.606 | 0.212 |
| | Within Groups | 13.200 | 20 | 0.660 | | |
| | Total | 17.440 | 24 | | | |
| Characteristic of honey type | Between Groups | 7.440 | 4 | 1.860 | 1.476 | 0.247 |
| | Within Groups | 25.200 | 20 | 1.260 | | |
| | Total | 32.640 | 24 | | | |
| Presence (strength) of aroma | Between Groups | 2..960 | 4 | 0.740 | 1.609 | 0.211 |

## IV. CONCLUSION

Based on the microbiological analyzes performed. all samples are correct and safe for consumption. The HMF analysis showed that the flower honey sample L1. from the Lukavac area does not meet the quality parameters prescribed by the Regulations on Honey and Other Bee Products and did not participate in the sensory evaluation. Other tested samples have a low HMF content. which confirms that they are of good quality.

Given that in the analyzed two types of honey from two different areas and it is to be expected that there is a difference in the sensory profile. and that it depends on climatic conditions, bee varieties, humidity and air temperature in the hive. processing and storage conditions. Sensory analysis showed that the forest honey K2 sample got the best and the K3 sample the worst rates. Analysis of variance showed that there is a statistically significant difference ($p < 0.05$) between the mean values for the sensory properties of purity and color.

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