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Influence of pH Value on HMF Content in Honey from Different Production Locations

Senada Suljkanović[1], Amir Zenunović[2]

[1]Inmer doo, Gradačac, Bosna i Hercegovina

[2]Faculty of Technology, Department of Agronomy / University of Tuzla, Bosnia and Herzegovina

Correspondence Author

Senada Suljkanović

senadadada95@gmail.com

Abstract: Beekeeping is an agricultural activity of special social, economic and ecological importance for the preservation of the country's biological diversity and the improvement of agricultural and fruit production. Honey is a bee product that has long been used as a food with a beneficial effect on the human body. The quality of honey is influenced by many factors such as: botanical origin of honey plants, nectar composition, geographical region where honey plants grow, climatic conditions, honey harvest season, time and method of storage, maturity and processing, presence of drugs, pesticides and microorganisms. Climate change cannot be influenced by beekeepers alone, but what they can do to create the preconditions for success is to follow the example of good beekeeping practice and be conscientious in their work. Although rising temperatures and prolonged heating create favorable conditions for the production of HMF, it has been proven that HMF is also formed when storing honey at lower temperatures, only at a much lower rate. In addition to the increase in temperature, the proportion of HMF in honey is significantly affected by the pH value of honey itself, because it has been proven that honey with lower pH has a higher proportion of HMF than honey with higher pH (Marinela et al. 2020).

Keywords: *beekeeping, honey, pH, HMF, location*

I. Honey

For centuries, honey has been used as food and natural medicine, and is defined as a natural sweet substance produced by honey bees (Apis mellifera) from the secretions of living parts of plants or from plant nectar and insect secretions that consume nectar plant juices by collecting, adding their own specific substances and store them in honeycombs to mature (Codex Alimentarius Commission, 2001).

In the last few decades, bees have been exposed to increased levels of pollution, which together with poor nutrition and pathogens contribute to the weakening of bee populations in Europe and the world. Various physiological disorders can occur as a consequence in the content of essential metals, whether it is a deficiency or a surplus. Other metals, such as cadmium, lead and mercury, do not have a physiological function and can

interfere with biological processes by interacting with macromolecules, replacing or otherwise affecting essential metals (Buchwalter, 2008).

1.1. Definition of honey

According to the Ordinance on the quality of honey and other bee products, honey is a sweet, thick, viscous, liquid or crystallized product produced by honey bees from nectar of honey plant flowers or honey dew, which bees collect, add their own specific substances and deposit in honeycomb cells. to mature (Ordinance on the quality of honey and other bee products, 2000). It is a real treasure of fructose and glucose, and traces of maltose, proteins, amino acids, vitamins (A, B, C, D, K) and minerals (Na, K, Ca, Mg, Co, Ni, Fe). Due to its easy digestibility, it restores lost energy, and with long-term use it ensures physical endurance and mental stability. Its properties make it an indispensable food in a healthy diet.

1.2. Creating honey

The basic raw material for honey production is nectar produced by various plants using the nectar glands, on their flowers or outside them. According to the chemical composition, nectar is a sweet fragrant liquid containing 50-75% water, sugars (sucrose, glucose and fructose), minerals, proteins, essential oils, vitamins, etc. The ratio of individual sugars in nectar depends on the type of plant, climate and other conditions. . The specific weight of honey ranges from 1.02 to 1.35, while the pH value is between 2.7 and 6.4. Nectar, which has 50% sugar, is best for bees. Bees prefer to collect nectar consisting of a mixture rather than just one sugar. The optimum temperature for nectar secretion is between 10 and 30 ° C, and the humidity is best between 60 and 80%. The conversion of nectar into honey is a complex physiological, chemical and physical process. All bee community workers take part in it. In addition to nectar, bees also collect sweet substances from the surface of leaves and needles. These sweet substances are called honeydew. Some plant lice (genera Aphidae and Lachnidae) expel honeydew from their body as excess food by feeding on the plant's juices. Its specific gravity is 1.0-1.3, while the pH is 5.1-7.9. The amount of carbohydrates is large and makes up 90-95% of the dry matter. The honeydew contains a number of sugars such as: sucrose, glucose, fructose, maltose, melicitose, mannose, etc. Bees lick the honeydew and transfer it to the hive for further processing. Whether bees collect honeydew depends on the lack of nectar in their area of flight, the amount of honeydew in nature, its chemical composition, atmospheric conditions, the strength of the bee community, etc. (Laktić and Šekulja, 2008). Bees suck nectar and honeydew into the honey bladder and bring it to the hive. Both sugars contain a lot of water and are not suitable for storage in honeycomb cells. The honeycomb wall is not permeable to water. For this reason, the bee removes excess water by transferring nectar and honeydew from cell to cell, and then uses enzymes from its body (invertase) to convert sucrose into glucose and fructose. The consequence of these processes is a higher concentration of sugar, which prevents the development of some microorganisms, and also prevents fermentation processes during honey storage. After ripening, honey should not contain more than 20% water. The bees then coat it in a honeycomb with a wax cover to preserve it as well as possible. Just before closing the honeycomb with a wax lid, the bee injects formic acid from the extremity glands into each honeycomb. Namely, formic acid serves as disinfectant. From the honey stored in this way, liquid honey is obtained after opening the lid and placing the honeycombs in centrifuges (Olaitan PB, 2007).

1.3. Types of honey

Codex standard defines honey as a natural sweet substance produced from honey nectar or secretions of living parts of plants, ie secretions of insects that suck sap from living parts of plants, honey bees (Apis mellifera), by collecting and modifying them by adding their own specific substances. they are disposed of, dried, stored and left in the honeycomb to mature (Codex Alimentarius Commission 2001).

Honey is divided into several basic types:

a) by origin:

flower or nectar honey obtained from plant nectar and

- honeydew or honeydew, which is obtained mainly from the secretions of insects (Hemiptera) that live on living parts of plants or from the secretions of living parts of plants.

b) according to the method of production and / or presentation:

- honey in honeycombs which bees in cells of freshly built honeycomb without brood or in hourly bases made exclusively of beeswax, sold in covered honeycomb or in parts of such honeycomb,

- honey with honeycomb or honey with parts of honeycomb is honey that contains one or more pieces of honey in the honeycomb,

- squeezed honey obtained by squeezing an open honeycomb without a litter,

- pitted honey obtained by spinning (centrifuging) an open honeycomb without a litter,

- pressed honey obtained by pressing honeycombs without litter, with or without the use of a moderate temperature not exceeding 45 ° C, and filtered honey is honey obtained by the process of removing foreign inorganic or organic substances as a result of a significant reduction in the content of pollen in honey.

1.4. Production conditions

In the last few years, primarily bad climatic conditions have significantly affected the productivity of bee colonies, which has significantly reduced the production of honey and other bee products, and this market has become very scarce in terms of honey varieties. When this period lasts longer, human intervention is necessary in terms of quality nutrition of bee colonies. This is supported by the fact that monocultures are used in intensive agriculture, which cannot meet the nutritional needs of bees in the right way (Naug, 2009). According to White et al. (1980) Honey is an essential carbohydrate material in which more than 95% is sugar, mostly glucose and fructose, as well as 22 more complex sugars in smaller quantities. Of all the sugars, only 7 are sweet and interesting to the bee (Frisch, 1934). According to Herbert et al. (1978) bees fed honey, invert syrup and corn syrup preferred food with a higher sucrose content. Also, according to Ivanov (1995), the yield of honey was the highest in bee colonies that were fed with invert syrups, except with invert syrup (citric acid), and all compared to refined sucrose. Based on research by Dustmann et al. (1995) it can be concluded that the difference in the sugar spectrum in bee food occurs very quickly after food intake, thanks to the effect of the enzyme invertase. In addition to the composition and properties of food in the hive, the amount of food is important for the development of bee colonies, and a positive correlation was found between the amount of honey in the bee colony and the mass of three-day-old larvae (Waller, 1972). Also, according to Schmickl and Crailsheim (2004), larvae and adult bees are highly dependent on the amount of food in the hive, with adult bees having to adjust their collecting activities and brood feeding activity, thus developing the so-called protection strategy according to appropriate needs and quality supply of carbohydrates and proteins.

The metal content in honey is largely determined by its botanical and geographical origin. As bees are exposed to pollutants located on an area of about 7 km2 in the vicinity of the apiary come into contact with air, soil and water, honey is expected to be a useful indicator of heavy metal pollution. Heavy metals come from industry, traffic, the use of cadmium-containing fertilizers, as well as mercury and arsenic-based pesticides, which are still used in some countries. As far as honey and bee products are concerned, the situation is similar: contamination comes from the environment, but also due to beekeeping activities such as smoking bees in the hive while working with them or removing honey extensions for honey extraction (Marina et al., 2020).

II. pH

The pH value of honey is of great importance during extraction (boiling) and storage of honey due to its effect on the texture, stability and shelf life of honey. Published papers suggest that the usual pH value of honey is

between 3.2 and 4.5. Knowing the pH value helps to discover the origin of honey as nectar or honeydew. The low pH value in honey prevents presence and growth of microorganisms. The pH value of honey also indicates the origin of honey. The acidity of different types of honey has a value in the range of 17.1-48 meq / kg. It comes from the presence of organic acids, proportional to the corresponding lactones or esters, as well as some inorganic ions (phosphate and sulfate ions). Variation in acidity between different types of honey it is caused by their different botanical origins as well as the difference in grazing seasons (Feas et al.,2010).

III. HMF

HMF is a cyclic aldehyde that can be formed by dehydration of fructose and glucose in an acidic medium, or in Maillard reactions (Tosi et al., 2004). HMF is further broken down into levulinic and formic acid, and the higher the reaction temperature, the faster the reaction. The proportion of HMF was once used as an indicator of honey forgery by adding invert sugar syrup. But it has been shown that honeys heated to higher temperatures also show a higher proportion of HMF. Therefore, high concentrations of HMF have been linked to inappropriate storage of honey at higher temperatures (Azeredo et al., 1999). Despite this, extremely high levels (above 100 mg / kg) can still be an indicator of honey counterfeiting. The appearance and content of HMF in honey also depend on the type of honey, its pH value, acid and moisture content, and light exposure. (Spano et al., 2005)

IV. Material and Methods

Honey samples were collected from honey producers (beekeepers) in the period of January 2021. The collected samples were produced in the spring / summer season of 2018, 2019, and 2020.

Such samples were delivered to the laboratory in glass containers in the amount of 250 g, with data on regional and botanical origin. A total of nine (9) samples of honey were collected, of which: three samples of meadow honey from 2018, three samples of meadow honey from 2019 and three samples of meadow honey from 2020. The samples are from different localities: Gradačac, Lukavac and Brčko.

Figure 1 Honey samples for analysis

1.5. Ph

The prepared sample is titrated in the presence of phenolphthalein with 0,1 mol / l sodium hydroxide solution until a light pink color appears.

Principle

The sample should be titrated to a light pink color with a solution of (NaOH) concentration of 0,1 mol / L.

Apparatus and accessories

The usual laboratory equipment is used to determine the degree of acidity.

Reagents

• Sodium hydroxide solution, c (NaOH) = 0.1 mol / l (without carbonate):

• 1% solution of phenolttalein (m / V) in ethanol, neutralized:

• CO2-free distilled water, obtained by boiling and then cooled.

Determination:

• Weigh 10 g of the sample and dissolve 75 ml of distilled water

• Titrate the prepared sample with 0.1 mol solution (NaOH) / l, with four to five drops of phenolphthalein as indicator. At the end of the color titration, 10 seconds must be maintained.

• For dark samples, a smaller amount of sample is weighed.

• Alternatively, a pH meter can be used and the sample titrated to pH - 8.3.

Calculation:

Acidity is expressed in millimoles of acid / kg and is calculated by the formula:

$$\text{Acidity} = 10 \times V$$

where is:

V = number of ml spent 0.1 mol (NaOH) / l to neutralize 10 g of honey.

Figure 2 pH and electrical conductivity reading

1.6. HMF

The method determines the concentration of 5-hydroxymethyl-furan-2-carbaldehyde (HMF), and the results are expressed in mg / kg. The determination is based on UV absorption of HMF at a wavelength of 284 nm. In order to avoid interference of other components absorbing at that wavelength, the absorbance of the sample solution and the standard solution is determined. The sample solution is an aqueous solution of honey, while the standard solution is an aqueous solution of honey to which sodium bisulfite has been added. The difference between the absorbance values of the standard solution and the sample solution represents the absorbance of the sample. The proportion of HMF in honey is obtained after the difference between the absorbance value at 336 nm and the value at 284 nm. The method is applicable to all honey samples (Bogdanov, 2009).

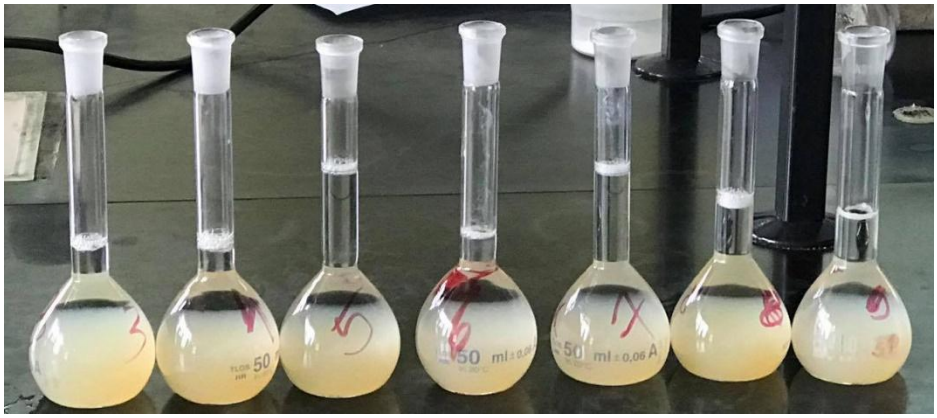

Figure 3 Samples ready for reading the absorbance on a spectrophotometer for HMF determination at two different wavelengths

Procedure:

Weigh 5 g of honey and dissolve with 25 ml of distilled water. Quantitatively transfer the solution to a 50 ml volumetric flask, add 0.5 ml of Carrez 1 solution and mix, then add 0.5 ml of Carrez 2 solution, mix again and make up to the mark with distilled water. Filter the contents of the flask through filter paper (shown in Figure 5). Weigh 5 ml of the filtrate into each of the test tubes. Add 5 ml of water to one test tube and mix the contents, and to the other add 5 ml of 0.2% Na-bisulphite and mix. The result is obtained by comparing the absorbance of the solution with the sample of the reference solution at 284 and 336 nm in a quartet cuvette of 10 mm for 1 hour.

Figure 4 Spectrophotometer for HMF

V. Results and discussion

Data collection was performed on the basis of analysis of honey from three localities in northeastern BiH (Lukavac, Brčko - Grčica and Gradačac). The analysis covers three time periods (2018-2020). The results were

classified according to factors affecting honey quality in ten categories: water activity, ph value, electrical conductivity, refractive index (22 ° c), HMF (mg / kg), Cd content (mg / kg), moisture content) - drying method and ash content (mg / 100 g). The sample weight of each analyzed honey is 5 grams. In appropriate sample was selected for this research, ie the available individuals from three areas were examined. The research units were honey producers, and one type of this honey was analyzed, more precisely meadow honey. The analysis of the obtained data was performed in the statistical program IBM SPSS 21.

The analysis of the obtained research results included a descriptive analysis of the research results. Descriptive analysis included mean (median, standard deviation), minimum and maximum values, and range of variation. The arithmetic mean gives us a number that is often considered the closest in theoretical and practical terms. On the other hand, the standard deviation tells us how close it is, or how much it deviates from the mean. The smaller the standard deviation, the closer the arithmetic mean is to the data. If the standard deviation is equal to 0, all values are the same, and the arithmetic mean is equal to all values (Tadić T., approach: 29.03.2020). The median is a number that divides numerical data into two equal sets - those that are (strictly) smaller and those that are (strictly) larger than the median (Ibid). Variance is used as a measure of the variability of a variable.

Basically, it represents the expected deviation of a random variable from its mean value. The analysis in the tables is divided into two bases. In the first part, physical and chemical parameters are presented on the basis of all analyzed places of the sample (Lukavac, Brčko-Grčica and Gradačac), by years. In the second part, the parameters related to individual sampling points for all three analyzed years are presented (2018, 2019, 2020).

1.7. pH value

Laboratory analysis of the pH value of the sample showed the following values:

Table 1 pH value of analyzed samples

1.	2.	3.	4.	5.	6.	7.	8.	9.
Lukavac 2018	Brčko – Grčica 2018	Gradačac 2018	Lukavac 2019	Brčko – Grčica 2019	Gradačac 2019	Lukavac 2020	Brčko Grčica 2020	Gradačac 2020
4,55	3,17	2,74	3,42	3,45	2,84	2,89	2,99	3,05

Table 2 Dependence of pH on the sampling site in 2018

Location of beehives	pH value
Lukavac	4.55
Brčko – Grčica	3.17
Gradačac	2.74

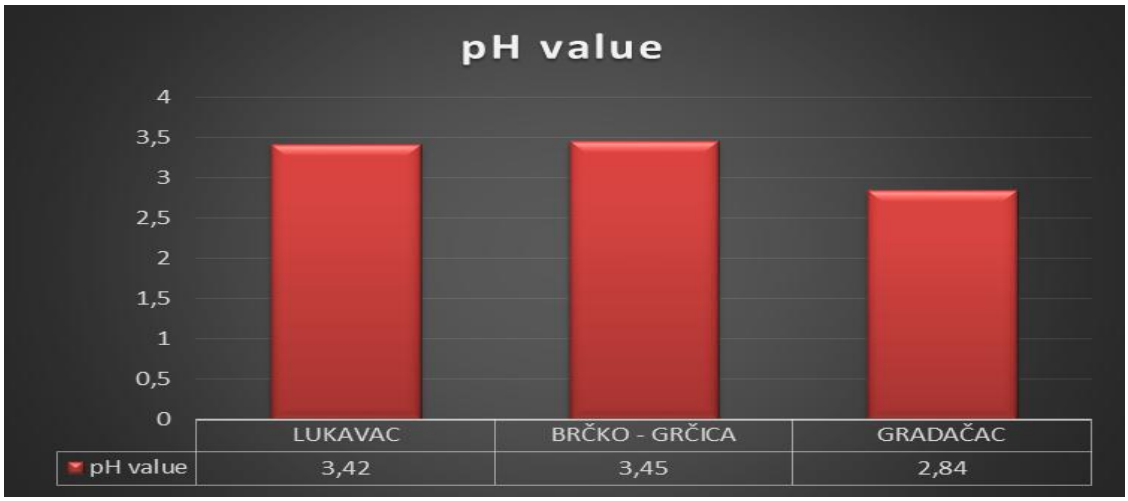

Figure 5 pH value - 2018

When it comes to pH value as a physico-chemical parameter, different values of all statistical indicators are observed. Thus, the arithmetic mean for 2018 is 3.4866667, while the standard deviation is very close to 1, and amounts to 0.945639114. Also, the calculated variance is 0.894, and a slightly larger range of variation of 1.81

Figure 6 pH value for 2018 for all analyzed areas

Arithmetic mean		3,48666667
Median		3,17000000
Standard deviation		0,945639114
Variance		0,894
Range of variation		1,810000
Minimum		2,740000
Maximum		4,550000
Percentiles	P 25	2,74000000
	P 50	3,17000000
	P 75	.

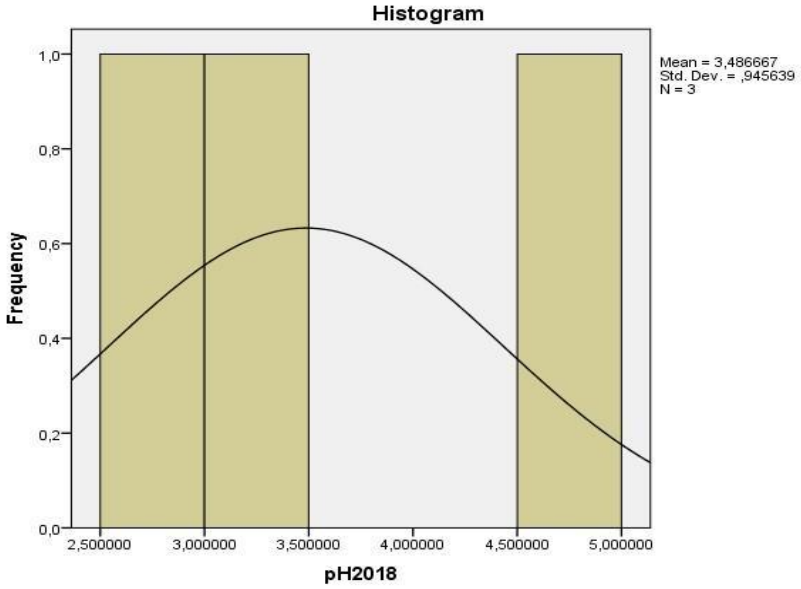

Figure 7 Histogram - pH value 2018

Table 3 Dependence of pH on sampling site in 2019

Location of beehives	pH value
Lukavac	3.42
Brčko – Grčica	3.45
Gradačac	2.84

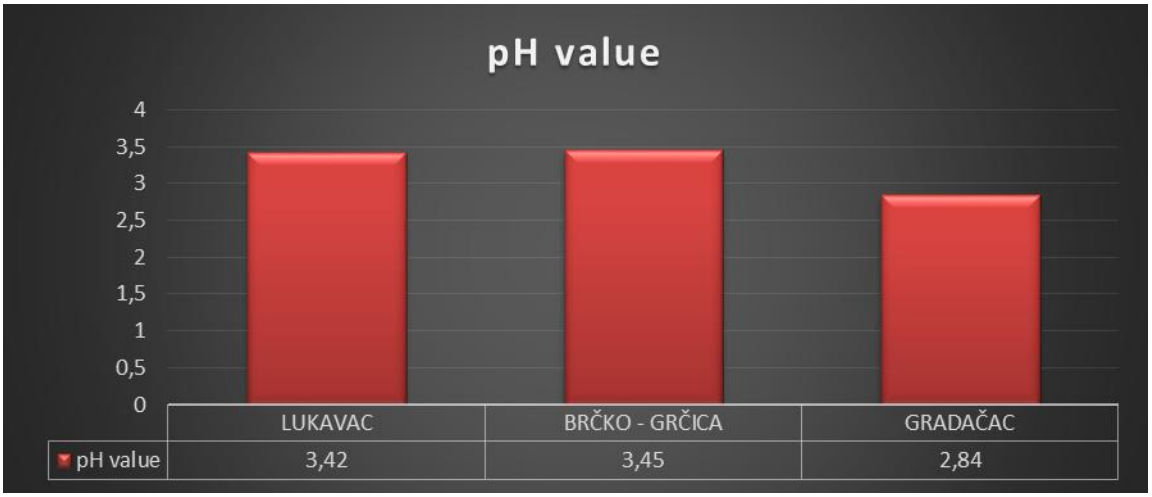

Figure 8 pH value - 2019

When observing the data from 2019, a slightly smaller standard deviation (0.343850743) and variance (0.118) are recorded. Based on these data, we can conclude that the samples had smaller deviations from the baseline.

Table 4 pH value for 2019 for all analyzed areas

Arithmetic mean		3,23666667
Median		3,42000000
Standard deviation		0,343850743
Variance		0,118
Range of variation		0,610000
Minimum		2,840000
Maximum		3,450000
Percentiles	P 25	2,84000000
	P 50	3,42000000
	P 75	.

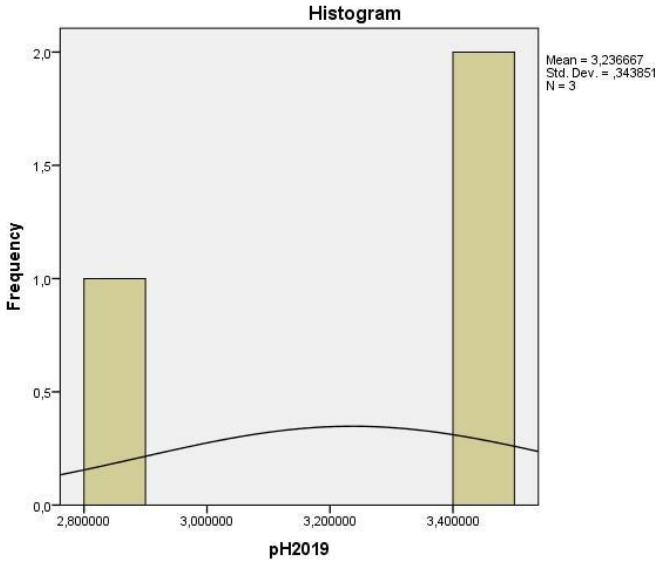

Figure 9 Histogram - pH value 2019

Table 5 Dependence of pH on the sampling site in 2020

Location of beehives	pH value
Lukavac	2.89
Brčko – Grčica	2.99
Gradačac	3.05

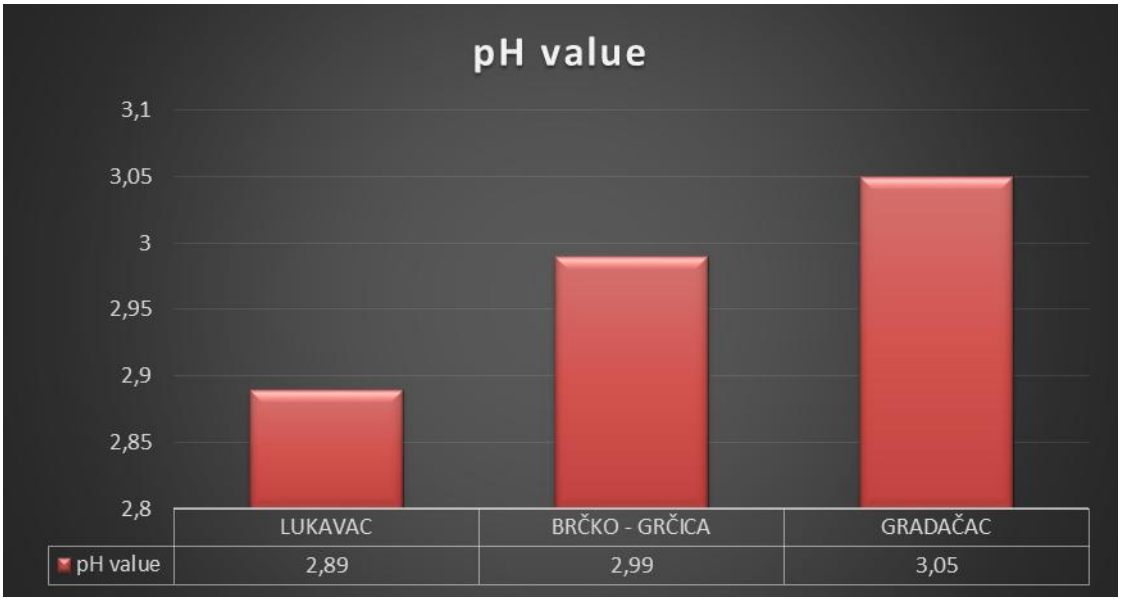

Figure 10 pH value – 2020

In 2020, we have the smallest deviation from the mean value, of 0.080829038, which is confirmed by the variance whose value is 0.007.

Table 36 pH for 2020 for all analyzed areas

Arithmetic mean		2,97666667
Median		2,99000000
Standard deviation		0,080829038
Variance		0,007
Range of variation		0,160000
Minimum		2,890000
Maximum		3,050000
Percentiles	P 25	2,89000000
	P 50	2,99000000
	P 75	.

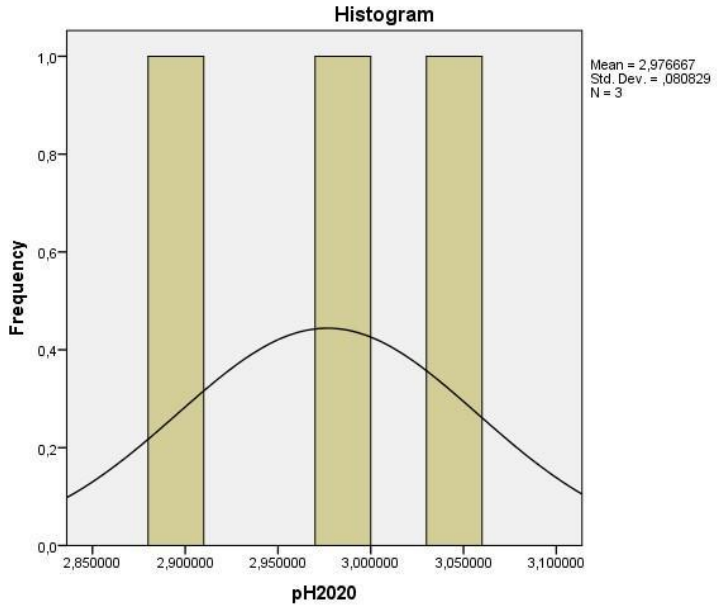

Figure 11 Histogram - Lukavac 2020

Table 6 pH dependence as a function of sampling year - Lukavac

YEAR OF SAMPLING	pH value	YEAR OF SAMPLING
2018	4.55	2018
2019	3.42	2019
2020	2.89	2020

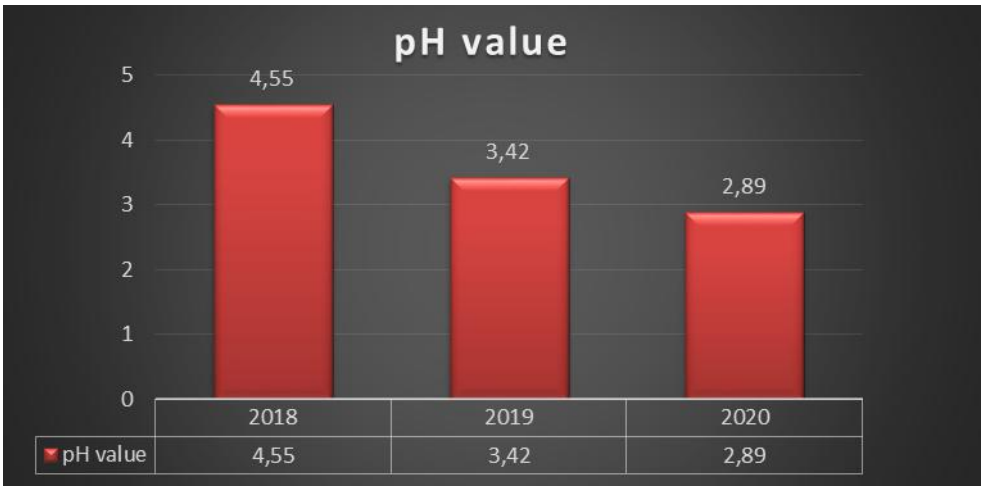

Figure 12 pH value - Lukavac

The analysis of all areas of the samples leads to the conclusion that the previous analysis was mostly influenced by the sample of honey taken from the area of Lukavac. As can be seen in the following table, this sample has the largest deviation, observing by years of 0.0847880, and a variance of 0.719.

Table 7 Lukavac pH value 2018-2020

Arithmetic mean		3,62000
Median		3,42000
Standard deviation		0,847880
Variance		0,719
Range of variation		1,660
Minimum		2,890
Maximum		4,550
Percentiles	P 25	2,89000
	P 50	3,42000
	P 75	.

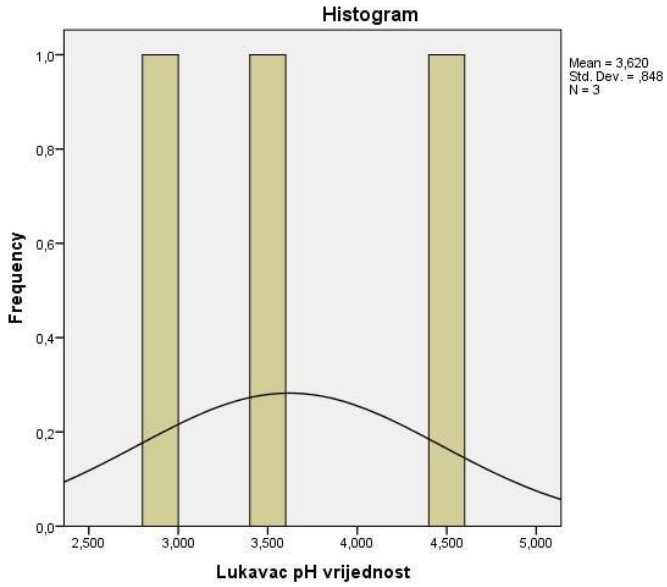

Figure 13 Histogram - pH value – Lukavac

Table 8 pH dependence as a function of sampling year - Brčko – Grčica

YEAR OF SAMPLING	pH value	YEAR OF SAMPLING
2018	3.17	2018
2019	3.45	2019
2020	2.99	2020

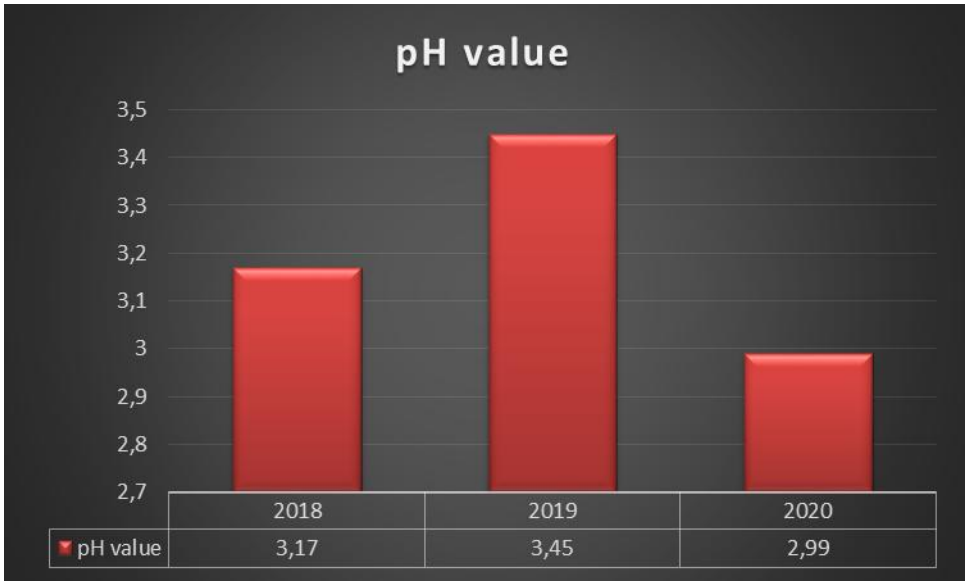

Figure 14 pH value - Brčko – Grčica

Samples from Brko and Gradačac do not have significant deviations.

Table 9 Brčko - Grčica pH value 2018-2020

Arithmetic mean		3,20333
Median		3,17000
Standard deviation		0,231805
Variance		0,054
Range of variation		0,460
Minimum		2,990
Maximum		3,450
Percentiles	P 25	2,99000
	P 50	3,17000
	P 75	.

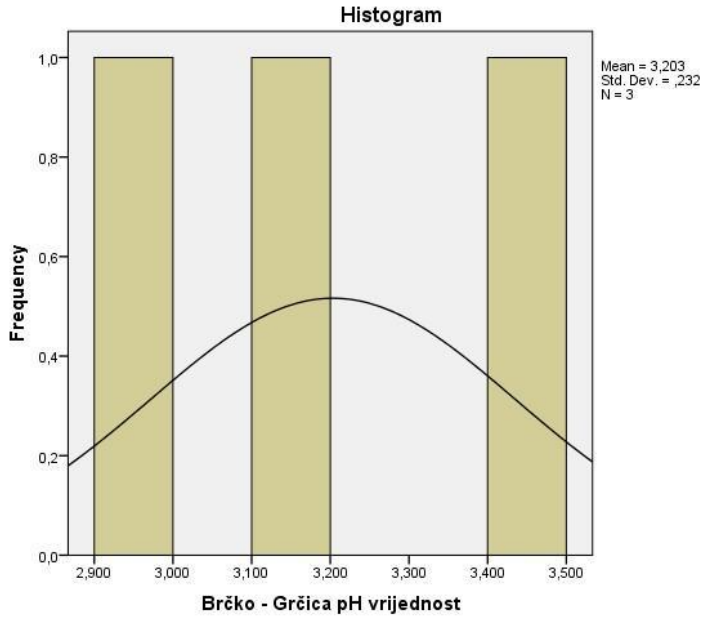

Figure 15 Histogram - pH value - Brčko - Grčica

Table 10 pH dependence as a function of sampling year - Gradačac

YEAR OF SAMPLING	pH value	YEAR OF SAMPLING
2018	2.74	2018
2019	2.84	2019
2020	3.05	2020

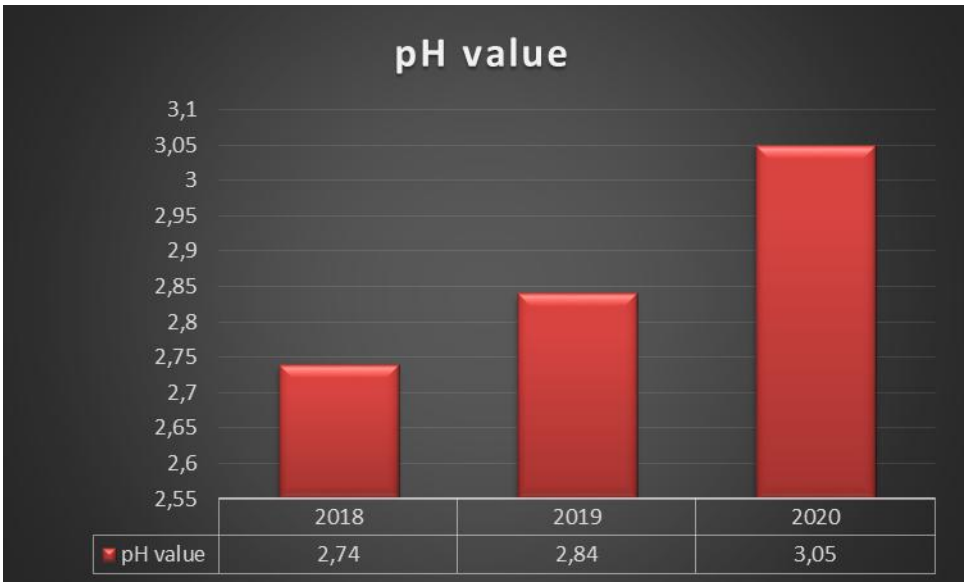

Figure 16 pH value - Gradačac

Table 11 Gradačac pH value 2018-2020

Arithmetic mean		2,87667
Median		2,84000
Standard deviation		0,158219
Variance		0,025
Range of variation		0,310
Minimum		2,740
Maximum		3,050
Percentiles	P 25	2,74000
	P 50	2,84000
	P 75	.

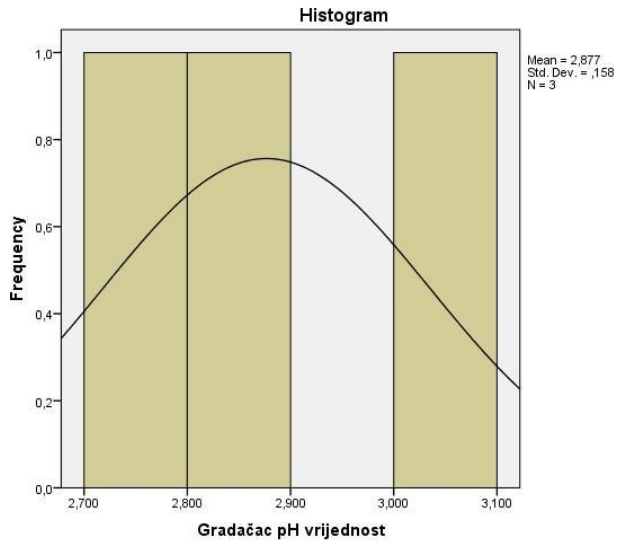

Figure 17 Histogram - pH value - Gradačac

Figure 18 pH value based on location and year of sampling

Sampling location	pH value	Year of sampling
Lukavac	4.55	2018
Brčko – Grčica	3.17	2018
Gradačac	2.74	2018
Lukavac	3.42	2019
Brčko – Grčica	3.45	2019
Gradačac	2.84	2019
Lukavac	2.89	2020
Brčko – Grčica	2.99	2020
Gradačac	3.05	2020

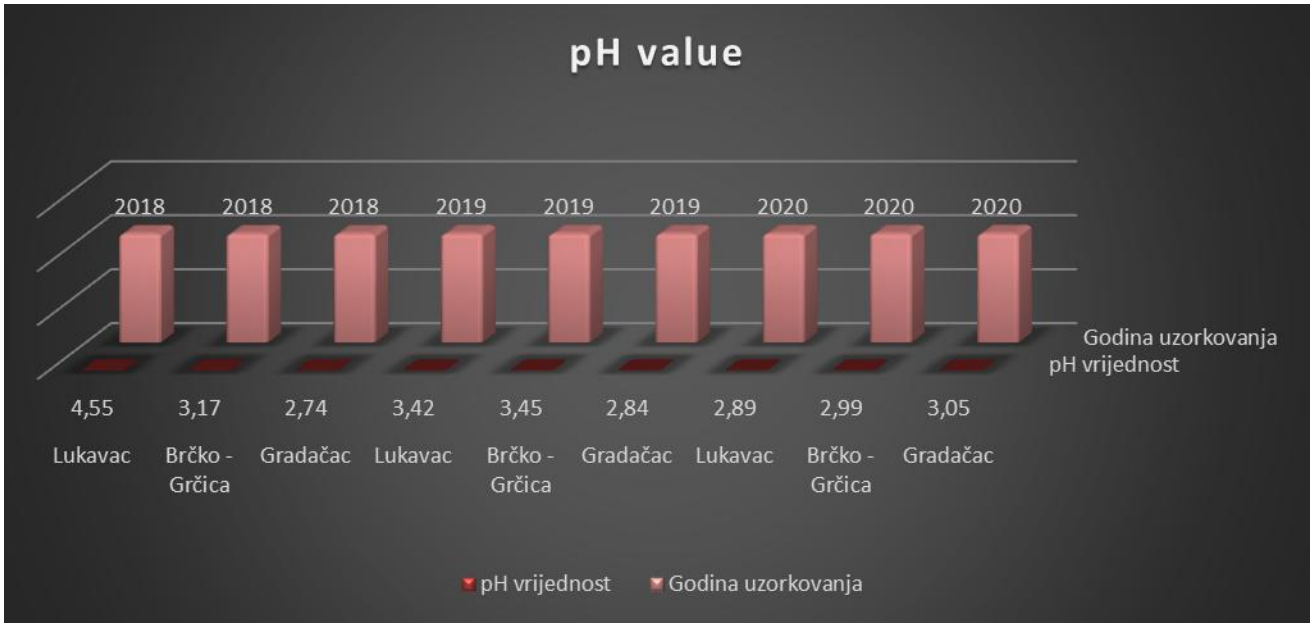

Figure 19 pH value based on location and year of sampling

Table 12 Descriptive statistics - pH value

	N	Minimum	Maximum	Mean	Std. Deviation
Lukavac pH value	3	2.890	4.550	3.62000	.847880
Brčko - Grčica pH value	3	2.990	3.450	3.20333	.231805
Gradačac pH vrijednost	3	2.740	3.050	2.87667	.158219
Valid N (listwise)	3				

VI. HMF (mg / kg)

Laboratory analysis of the Hydroxymethylfurfural (HMF) sample showed the following values:

Table 13 HMF analyzed samples

1.	2.	3.	4.	5.	6.	7.	8.	9.
Lukavac 2018	Brčko – Grčica 2018	Gradačac 2018	Lukavac 2019	Brčko – Grčica 2019	Gradačac 2019	Lukavac 2020	Brčko Grčica 2020	Gradačac 2020
6,1377	17,5149	38,7723	44,4609	74,5506	80,8380	17,2155	74,2512	37,4250

When it comes to HMF, as a physico-chemical parameter, from statistical analysis, we can conclude that the samples deviated significantly from the average values. Thus, for 2018, the standard deviation of 16.5646956 and the variance of 274.389 were calculated, which leads to the conclusion that the samples are small large deviations from the mean values.

Table 14 Dependence of HMF (mg / kg) on the sampling site in 2018

Location of beehives	HMF (mg/kg)
Lukavac	6.1377
Brčko – Grčica	17.5149
Gradačac	38.7723

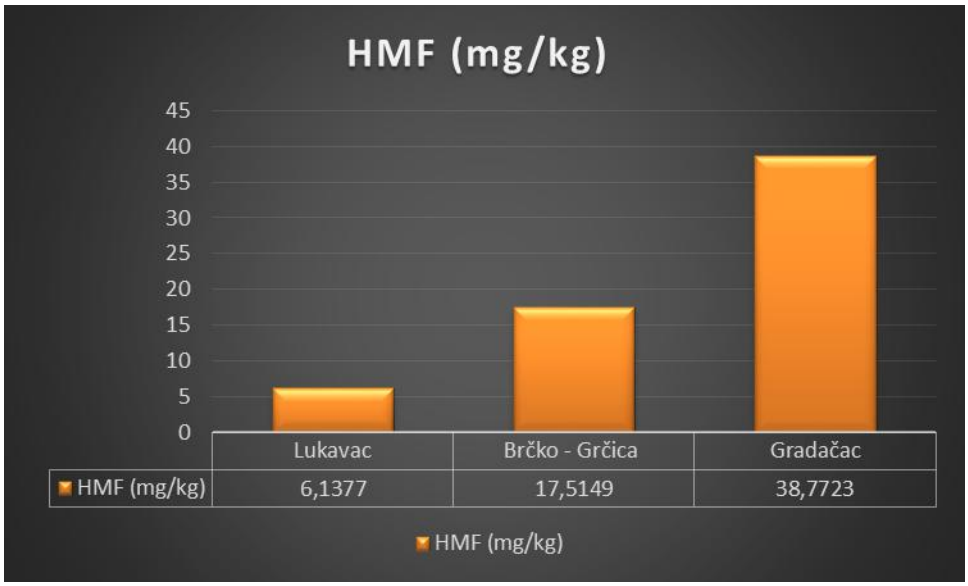

Figure 20 HMF - 2018. Year

Table 15 HMF for 2018 for all analyzed areas

Arithmetic mean		20,808300
Median		17,514900
Standard deviation		16,5646956
Variance		274,389
Range of variation		32,6346
Minimum		6,1377
Maximum		38,7723
Percentiles	P 25	6,137700
	P 50	17,514900
	P 75	.

Graphically, the analysis looks like this:

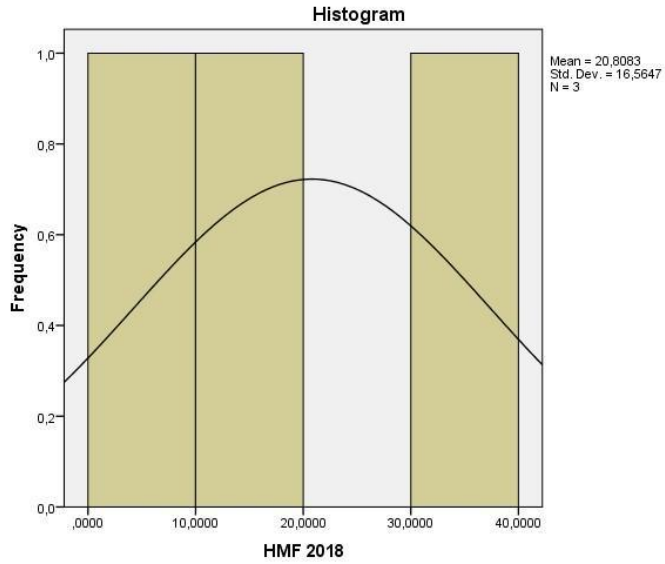

Figure 21 Histogram - HMF 2018

In 2019, the deviation increased, and the standard deviation is 19.4431430, while the variance is 378.036.

Table 63 Dependence of HMF (mg / kg) on the sampling site in 2019

Location of beehives	HMF (mg/kg)
Lukavac	44.4609
Brčko – Grčica	74.5506
Gradačac	80.838

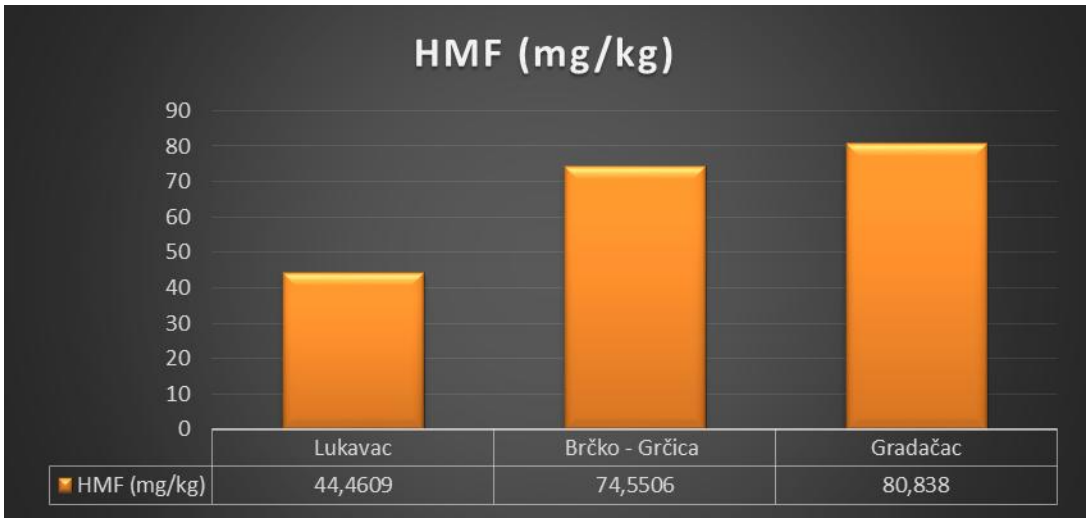

Figure 22 HMF - 2019. Year

Table 16 HMF 2019 for all analyzed areas

Arithmetic mean		66,616500
Median		74,550600
Standard deviation		19,4431430
Variance		378,036
Range of variation		36,3771
Minimum		44,4609
Maximum		80,8380
Percentiles	P 25	44,460900
	P 50	74,550600
	P 75	.

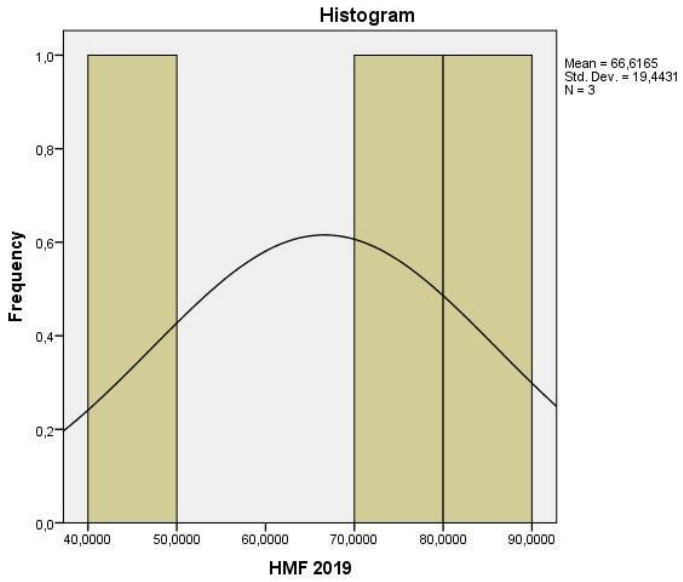

Figure 23 Histogram - HMF 2019

Table 17 Dependence of HMF (mg / kg) on the sampling site in 2020

Location of beehives	HMF (mg/kg)
Lukavac	17.2155
Brčko – Grčica	74.2512
Gradačac	37.425

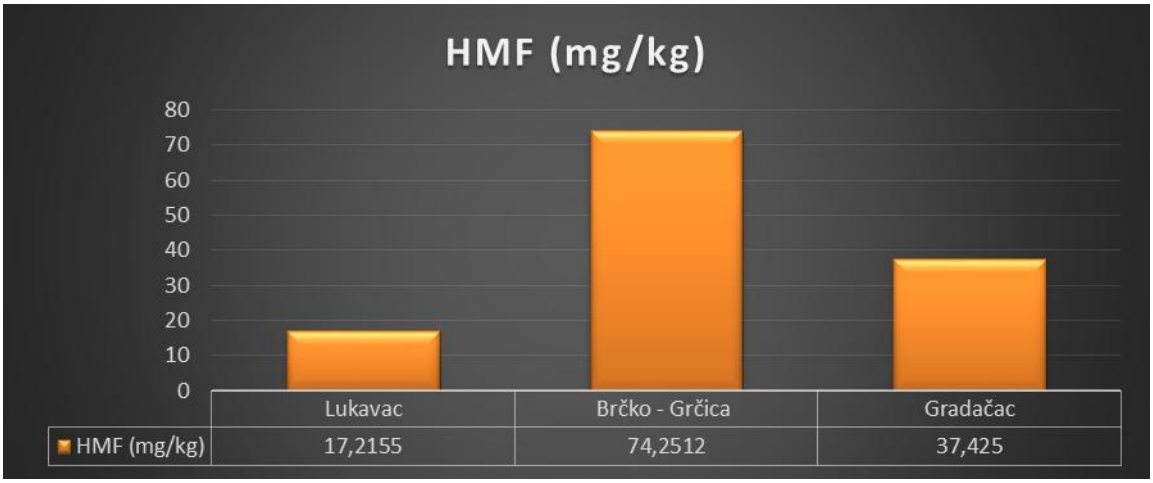

Figure 24 HMF - 2020. Year

Table 18 HMF for 2020 for all analyzed areas

Arithmetic mean		42,963900
Median		37,425000
Standard deviation		28,9184600
Variance		836,277
Range of variation		57,0357
Minimum		17,2155
Maximum		74,2512
Percentiles	P 25	17,215500
	P 50	37,425000
	P 75	.

The largest deviations were recorded in 2020, where the standard deviation is 28.91846, while the variance is 836.277.

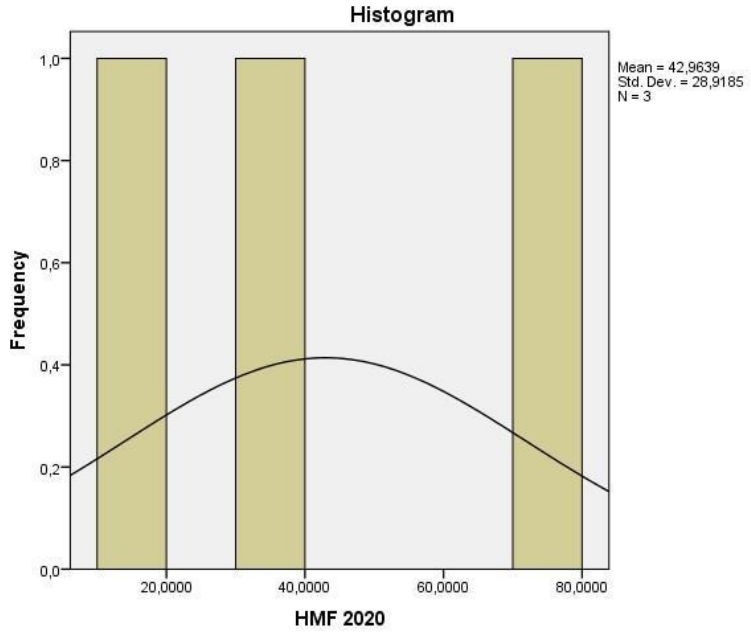

Figure 25 Histogram - HMF 2020

Table 19 Dependence of HMF (mg / kg) as a function of sampling year - Lukavac

Year of sampling	HMF (mg/kg)	Year of sampling
2018	6.1377	2018
2019	44.4609	2019
2020	17.2155	2020

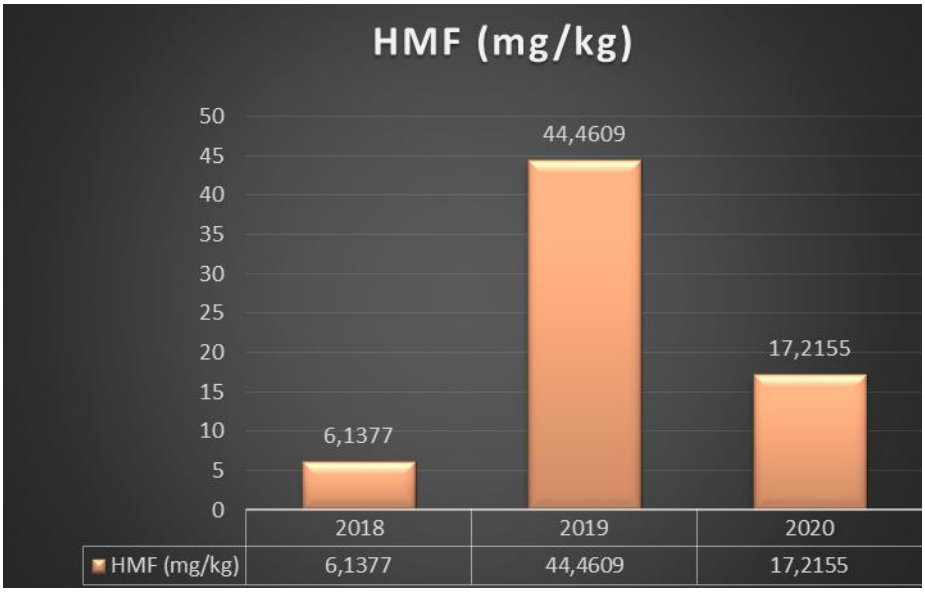

Figure 26 HMF - Lukavac

Table 20 Lukavac - HMF (mg / kg) 2018-2020

Arithmetic mean		22,6047000
Median		17,2155000
Standard deviation		19,72180321
Variance		388,950
Range of variation		38,32320
Minimum		6,13770
Maximum		44,46090
Percentiles	P 25	6,1377000
	P 50	17,2155000
	P 75	.

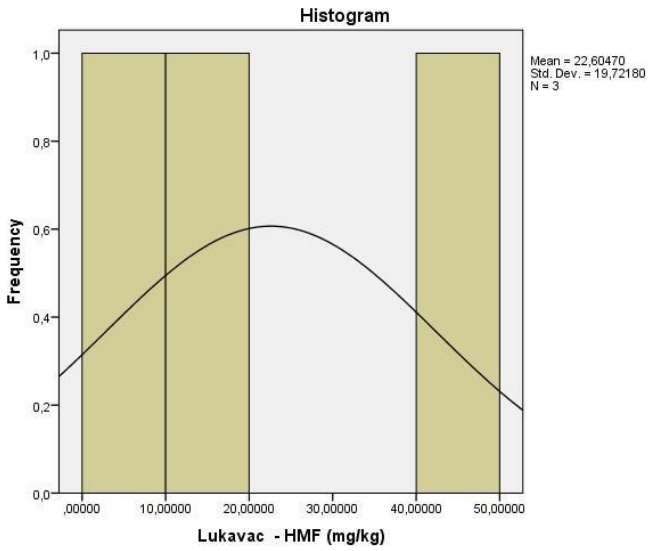

Figure 27 HMF Lukavac

Table 21 Dependence of HMF (mg / kg) as a function of sampling year - Brčko - Grčica

Year of sampling	HMF (mg/kg)	Year of sampling
2018	17.5149	2018
2019	74.5506	2019
2020	74.2512	2020

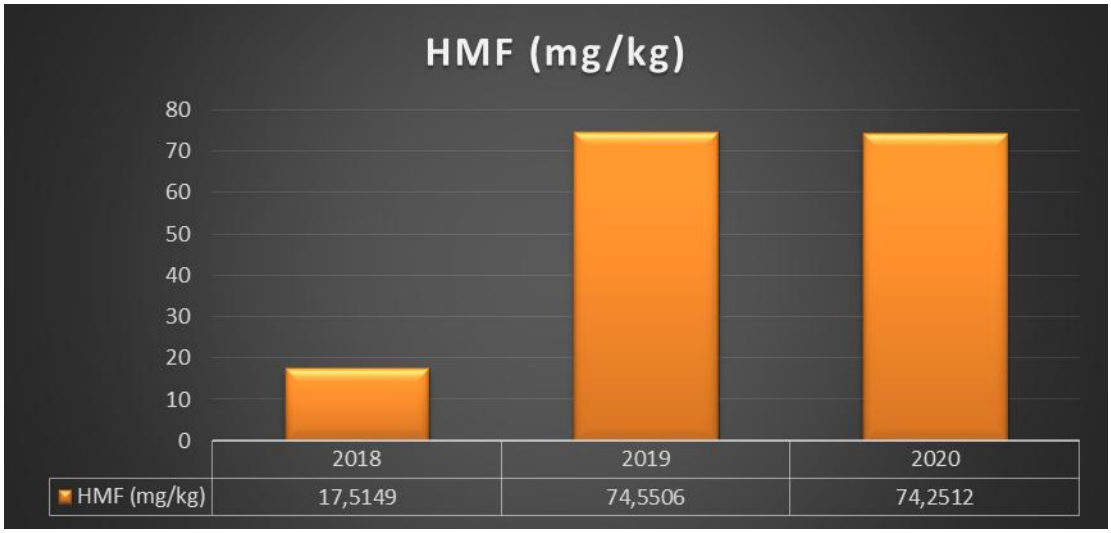

Figure 28 HMF - Brčko - Grčica

Table 22 Brčko Grčica - HMF (mg / kg) 2018-2020

Arithmetic mean		55,4389000
Median		74,2512000
Standard deviation		32,84348858
Variance		1078,695
Range of variation		57,03570
Minimum		17,51490
Maximum		74,55060
Percentiles	P 25	17,5149000
	P 50	74,2512000
	P 75	.

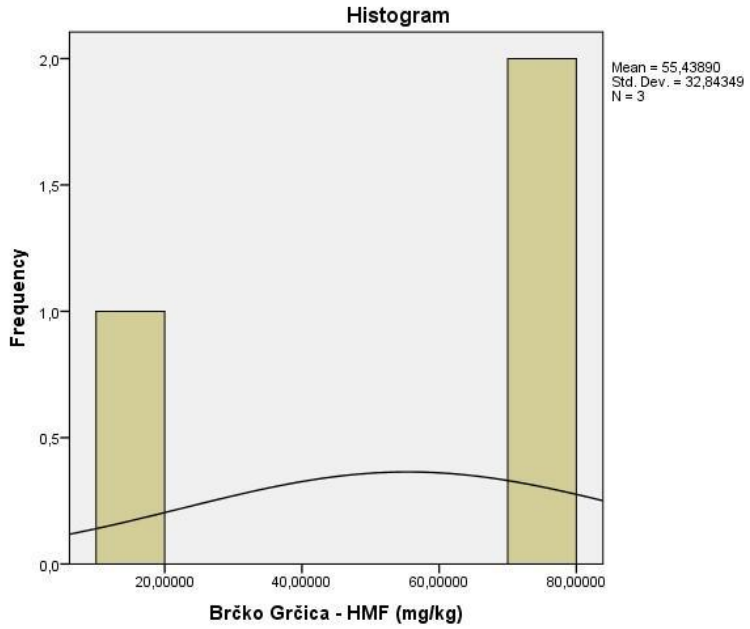

Figure 29 Histogram - HMF - Brčko - Grčica

Table 23 Dependence of HMF (mg / kg) as a function of sampling year - Gradačac

Year of sampling	HMF (mg/kg)	Year of sampling
2018	38.7723	2018
2019	80.838	2019
2020	37.425	2020

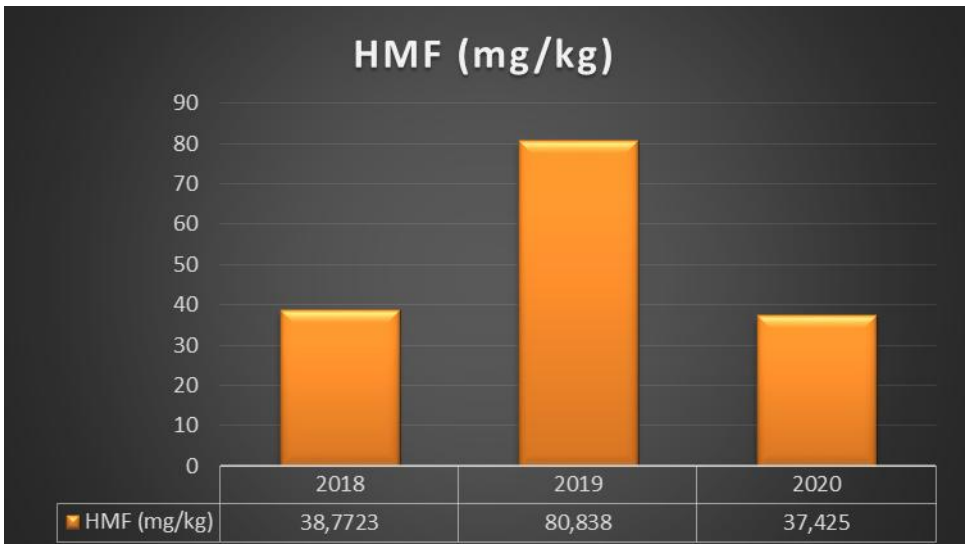

Figure 30 HMF – Gradačac

Table 24 Gradačac - HMF (mg / kg) 2018-2020

Arithmetic mean		52,3451000
Median		38,7723000
Standard deviation		24,68476893
Variance		609,338
Range of variation		43,41300
Minimum		37,42500
Maximum		80,83800
Percentiles	P 25	37,4250000
	P 50	38,7723000
	P 75	.

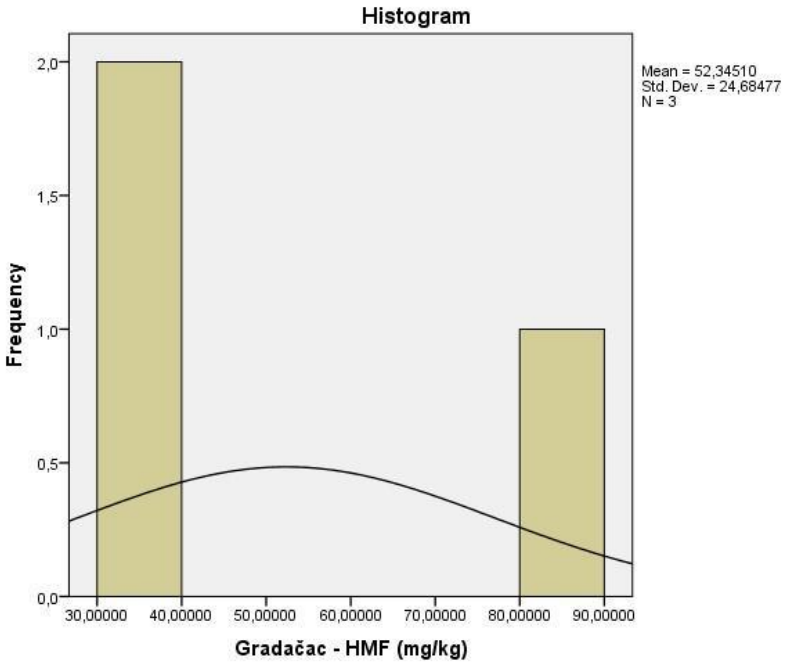

Figure 31 HMF - Gradačac

Table 25 HMF based on location and year of sampling

Year of sampling	HMF (mg/kg)	year of sampling
Lukavac	6.1377	2018
Brčko – Grčica	17.5149	2018
Gradačac	38.7723	2018
Lukavac	44.4609	2019
Brčko – Grčica	74.5506	2019
Gradačac	80.838	2019
Lukavac	17.2155	2020
Brčko – Grčica	74.2512	2020
Gradačac	37.425	2020

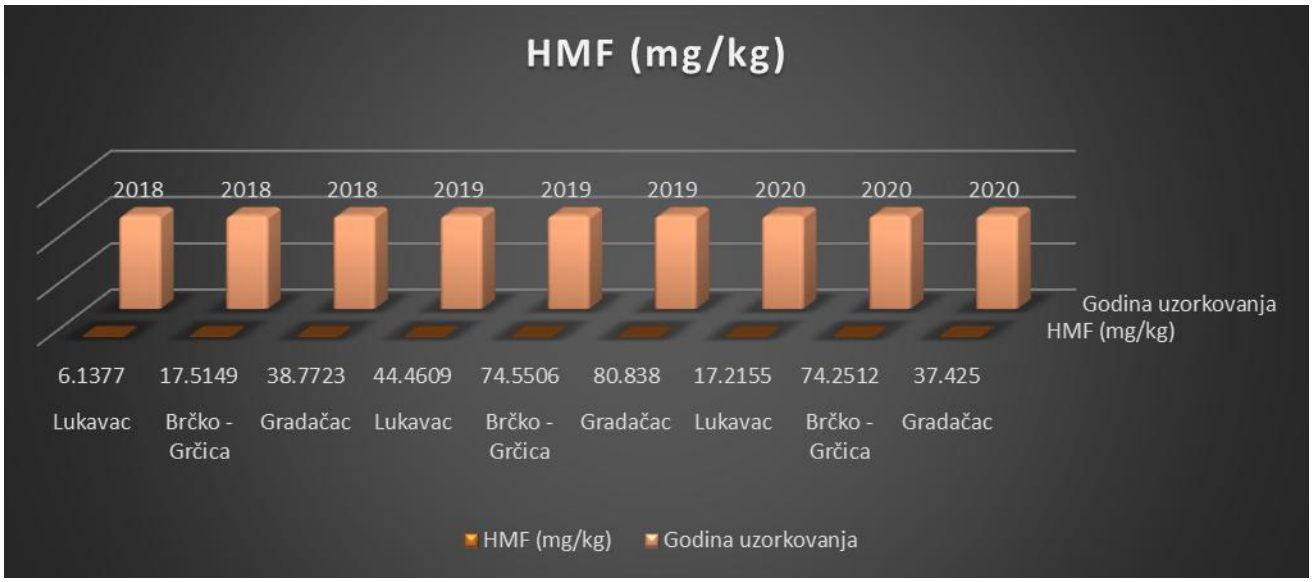

Figure 32 HMF based on location and year of sampling

Table 26 Desktop statistics - HMF

	N	Minimum	Maximum	Mean	Std. Deviation
Lukavac - HMF (mg/kg)	3	6.13770	44.46090	22.6047000	19.72180321
Brčko Grčica - HMF (mg/kg)	3	17.51490	74.55060	55.4389000	32.84348858
Gradačac - HMF (mg/kg)	3	37.42500	80.83800	52.3451000	24.68476893
Valid N (listwise)	3				

VII. Conclusion

In this research, we can state that the parameter "HMF", for all areas of analysis and all years of analysis, deviates from the basic set, ie the mean value, ie. the samples are different from the basic set during the analyzed 3 years.

Ačlso, it can be stated that the parameter "pH value", for all areas of analysis and all years of analysis, deviates from the basic set, ie from the mean value, ie. the samples are different from the basic set, which was mostly influenced by the sample from the area of Lukavac, which had the largest deviations during the analyzed 3 years.

With a range of 3.34 to 6.05 to conclude that honey is a relatively acidic product.

Storage temperature of honey should be carefully controlled (lower than 35°C) to preserve its chemical and sensory quality.

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