

## ANALYSIS OF BIOCHEMICAL COMPOSITION OF HONEY SAMPLES FROM BOSNIA AND HERZEGOVINA

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### ABSTRACT

In this work has been carried out analysis of the quality of honey from 18 different locations and different floral backgrounds in Bosnia and Hezegovina. It has been made analysis of only those known samples to beekeeper that produces and it has not done analysis of commercial kinds of honey. The analysis has been done according to the regulations on quality control of honey F. B&H which is in accordance with international standards. It has been analyzed the following parameters of honey: electrical conductivity of honey that was moving in the range from 0.13 to 1.4 mS / cm, by reducing sugars in the range from 64.8% to 85.00%, sucrose from 1.23% to 6%, insoluble matter from 0.12% to 0.5%, a water content from 15.4% to 10.4% and the acidity from 15-34 mmol acid / kg honey. The results have showed that in all samples of controlled parameters within the limits has been prescribed by the domestic and international standards of quality honey so that we can conclude that the beekeepers in B&H produce high quality honey.

**KEYWORDS:** Honey, Sugars, International Standards

### INTRODUCTION

Natural honey is one of them ostwidely sought products duet o its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of sub stances it contains. Codex Aliment arius Commission defined honey as the naturals, weet substance produced by honey bees, *Apismellifer a*, from then ectar of plants(blossoms)or from the secretion so fliving parts of plant so rexcretions of plantz sucking insects on the living part so f plants, which honey bees collect, trans form by combining with specific substances of the Irown, deposit, dehydrate, store and leave in the honey comb to ripen and mature(1,2).The bees are said to produce honey in order to serve as their source of food in times of scarcityor during harsh weather conditions(3).

Essentially, natural honey is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water,0.1–0.4% protein, 0.2%ashandminor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants (3-7). Each of the semin or constituents is known to have distinctive nutritional or medicinal properties and the unique blend accounts for the varied and different applications of natural honeys (3). Although them aj or constituents of honey are nearly the same in all honey samples, the precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees for age (3,8-11).Furthermore, differences in climaticconditionsandvegetationsareimportantfactorsthatcanaffect the various properties of honey. In Bosnia and Herzegovina due to climatic conditions present in a various fauna with a large number of endemic species and provides favorable conditions for dealing with beekeeping that gives to honey from this region

special characteristics.

## **MATERIALS AND METHODS**

### **Samples**

Samples of honey for analysis were collected on the area of the Federation of Bosnia and Herzegovina from different geographical areas and various botanical origin. It has been carried out analysis of 18 honey samples (Table 1.) taken from different individual honey producers - beekeepers. All analyzes in this work were made according to the regulation on methods for control of honey and other bee's products (12) which is in line with the Codex standard for honey and the harmonized with method of the European Commission for honey. It has been carried the following physical-chemical analyzes of honey: testing of electrical conductivity, the content of reducing sugars, content of sucrose, content of insoluble matter, refractive index / water content and acidity of honey.

### **Preparation of the Sample for Analysis**

Depending on the consistency of honey, samples for analysis have prepared in various ways. If honey is in the liquid state, before the start of the analysis, it has been gently mixed with wand or shaking. If honey has granulated, sealed container with the sample to be placed in a water bath and heated for 30 minutes at 60 ° C, if necessary at a temperature of 65 ° C. During the heating, honey can be mixed with the wand or circularly stir and then quickly cold. If honey contains unknown matters, such as wax, parts of bees or parts of the honeycomb, the sample is heated at a temperature of 40° C in a water bath and then strained through a cloth placed on the funnel heated hot water. If the honey is in honeycomb, the honeycomb opens, strained through a wire sieve with a square hole dimensions 0.5 x 0.5 mm. If part of the honeycomb and wax pass through a sieve the sample had been heated in a water bath at a temperature of 60° C, if necessary heated for 30 minutes and at a temperature of 65° C and during the heating stir with wand or shake with circular motions and then quickly to be cold.

If honey in the honeycomb has granulated it has heated to dissolve the wax, stirred and cooled. After cooling, the wax has removed.

### **Electrical Conductivity**

Electrical conductivity is a physical attribute that greatly depends on the content of minerals matter and acids in honey; if it is higher, the higher is the electrical conductivity of honey. Method is valid to determine electrical conductivity in honey in the range from 0.1 to 3 milliSiemens / cm (mS / cm-1). Electrical conductivity in honey has defined by using 20% of volumen of aqueous solution of honey at 20° C, where 20% has related to the dry matter of honey. The results have expressed in milliSiemens cm-1. The electrical conductivity of a solution that comprises 20 grams of dry matter of honey in 100 ml of distilled water has measured by using a cell electro conduct meter. Determination of electrical conductivity has based on measuring the electrical resistance, which is the reciprocal to electrical conductivity. The method has been based on the original work of Vorvohl (1964) (1-5).

### **The Content of Reducing Sugars**

The quantity and the relation between certain carbohydrates in honey depend primarily on its botanical and geographical origin, but also on the composition and intensity of nectar secretion, climatic conditions and physiological

state and breed of bees (13). Qualitative and quantitative determination of carbohydrates in honey is the subject of many studies whether that it was routine assessments of quality (according to the Regulations), the determination of adulteration of honey or determining the botanical and / or geographical origin of honey.

The principle of this method has based on the reduction of the Fehling's solution by litracionim solution of reduced sugar honey by using methylene blue as an indicator.

### **The Sucrose Content**

The ratio of fructose and glucose (F / G) is characteristic for certain types of honey and in most cases greater than 1.0 (14). As rich in fructose stand out honey of acacia and chestnut (F / G 1.5-1.7) while one of the few kinds with higher shares of glucose honey of oil turnip and dandelion (15). The ratio of fructose and glucose, and the ratio of glucose and water in honey are very important because they help can determine and predict the tendency of crystallization of honey. The main sugars provide sweetness of honey is fructose, glucose, sucrose and maltose.

Determination of sucrose is very important to determine the possible adulteration of honey, feeding the bees with sugar (sucrose) or direct adding of sugar in honey.

The principle of this method is based on the hydrolysis of sucrose reduction of Fehling's solution by titration with reduced sugars from the hydrolyzate of honey with methylene blue.

### **The Content of Insoluble Solids**

The share of mineral substances in honey largely depends on its botanical origin, but also on the climatic conditions and the content of soil on which grew honey plant. Characteristical soil of certain regions has reflected in the mineral composition of honey producing plants and mineral composition of its nectar and pollen (16).

Preparation of the sample: to be measured out 20 g of the sample with an accuracy of  $\pm 10$  mg, dissolved in a given quantity of distilled water at  $80^{\circ}\text{C}$  and mixed well. The prepared sample is first filtered through a dried and steady sintered funnel, pore size of 15 to 40  $\mu\text{m}$ . The residue ought to be washed with hot water ( $80^{\circ}\text{C}$ ) whereby sugar set free, which is determined by test. Funnel to be dry for one hour at a temperature of  $135^{\circ}\text{C}$ , to be cooled and measured with an accuracy of 0.1 mg. The amount of matter insoluble in water has been expressed as a percentage (m/m) and calculated by the following formula:

The percentage of insoluble matter in water =  $(100 \times \text{quantity of residue}) / (\text{a measured sample})$

The refractive index of water / water content

The measurement has carried out by refractometer which works on the principle of refraction of light when it passes through the solution. The measurement has carried out usually at  $20^{\circ}\text{C}$ , and the results obtained vary depending on the measurement temperature. Since the refractive indexes differ from honey measured for a solution of the same concentration of sucrose should be used for specific tables for this purpose. The principle of this method has based on determining the refractometer. In addition to the usual laboratory equipment has required refractor too.

Preparation of sample: The sample has prepared in the manner provided for the method of preparing a sample for analysis, and then the refractive index of the sample determined by refractometer at a constant temperature of  $20^{\circ}\text{C}$ . On

the basis of the refractive index has calculated amount of water, such as to the attached table. If the index has not determined by the temperature of 20° C, it is going to be taken into account the correction of the temperature and results that have been reduced to a temperature of 20° C.

### The Acidity of Honey

The prepared sample has titrated in the presence of fenolfialeina solution of 0.1 mol sodium hydroxide until appearance of light pink color. In determining of degree of acidity it has been used a common laboratory equipment. Reagents: Sodium-liidroksida. C (NaOH) - 0, 1 mol l (without carbonate); 1% solution of fenolttaleina (m) in ethanol neutralized by: distilled water without CO<sub>2</sub>, obtained by boiling and then cooled. Determination: to be measured out 10 g of the sample and dissolved in 75 ml of distilled water. The prepared sample was titrating with 0.1 mol solution (NaOH) and with four or five drops of fenolttaleina as indicators. At the end of titration color must last for 10 seconds. For dark samples it is measure the small quantity of sample. Alternatively it can be used pli-meter and titrating sample to pll - 8.3. Calculation of acidity has expressed in niilimolima acid kg and has calculated by using the formula.

Acidity = 10 x V wherein.

V - The number of spent ml 0.1 mol (NaOH) l for neutralization of 10 g of honey.

**Table 1: List of Samples**

Sample Number	Type and Origin of Honey	Location
1	acacia honey	Zavidovići
2	forest honey	Bugojno
3	meadow honey	Bugojno
4	meadow honey	Prusac
5	pine honey	Zavidovići
6	meadow honey	Zavidovići
7	meadow honey	Blidinje
8	heather honey	Tomislav Grad
9	heather honey	Mostar
10	meadow honey	Srebrenik
11	meadow honey	Srebrenik
12	chestnut honey	Serbrenik
13	pine honey	Prusac
14	chestnut honey	Cazin
15	pine honey	Bihać
16	meadow honey	Zenica
17	acacia honey	Zenica
18	acacia honey	Kakanj

## RESULTS AND DISCUSSIONS

### The Electrical Conductivity of Honey

From above results we can see that the highest conductivity shows forest and chestnut honey. Conductivity honey samples were ranging from 0.13 to 1.4 mS / cm which is within the normal range.

### The Content of Reduced Sugar

The obtained values for determining the content of invert sugar in the sample of honey has ranged from 64.8 to 85.00%. Therefore, we can conclude that the reduced sugar content expressed as invert to the extent prescribed by the

Regulations on quality of bee products or any pattern of honey not departs of the given value.

### The Sucrose Content

The obtained values for determining the content of invert sugar in the sample of honey were ranging from 64.8 to 85.00%. Therefore, we can conclude that the reduced sugar content expressed as invert to the extent prescribed by the Regulations on quality of bee products or any pattern of honey that do not departs of the given value.

**Table 2: Summary Results of Physical and Chemical Analysis of Honey**

Uzorak	Electrical Conductivity ms/Cm	Reduced Sugar (%)	Sucrose %	Insoluble Solids%	Water Content %	Acidity of Honey (Mmol.Kis/1 Kg Meda)
1.	0,2 mS/cm	72 %	3,6 %	0.2 %	17,2	22
2.	1,13 mS/cm	70%	4 %	0,4 %	19,00	28
3.	0,6 mS/cm	64,8 %	2,1 %	0,22 %	16,2	29
4.	0,25 mS/cm	78 %	4,3 %	0,3 %	15,4	32
5.	0,8 mS/cm	72,6 %	2,8 %	0,12 %	17,00	25
6.	0,13 mS/cm	80 %	4 %	0,32 %	17,8	17
7.	0,52 mS/cm	77,2 %	3,1 %	0,5 %	19,4	15
8.	0,62 mS/cm	76 %	6 %	0,32 %	20,2	24
9.	0,58 mS/cm	78 %	5,3 %	0,12 %	19,00	19
10.	0,22 mS/cm	84 %	2,5 %	0,14 %	15,4	28
11.	0,4 mS/cm	82,6 %	1,85 %	0,23 %	19,00	27
12.	1,2 mS/cm	79 %	3,1 %	0,15 %	15,8	15
13.	0,74 mS/cm	81 %	3,8 %	0,14 %	18,2	31
14.	1,4 mS/cm	80 %	2,6 %	0,25 %	17,4	13
15.	0,8 mS/cm	84 %	2 %	0,35 %	16,2	14
16.	0,6 mS/cm	83,6 %	3 %	0,38 %	20,6	17
17.	0,66 mS/cm	84 %	1,95 %	0,44 %	15,8	32
18.	0,76 mS/cm	85 %	1,23 %	0,20 %	20,6	34

### The Content of Insoluble Solids

The obtained values for determining the content of substances insoluble in water in a sample of honey were ranging from 0.12 to 0.5%, which is qualified in accordance with the rulebook on the quality of bee products.

### Refractive Index / Water Content

The measurement has carried out with refractometer which works on the principle of refraction of light when it passes through the solution. The measurement is carried out usually at 20° C, and the results obtained vary depending on the measurement temperature. Since the refractive indices of honey differs from that measured for a solution of sucrose with the same concentration it must be used special table for this purpose

The water content in the samples from the Federation BiH were ranging from 15.4% to 10.4% and is in line with the rules on the permitted amount of water in honey and bee products.

### The Acidity of Honey

The values of the acidity of honey in our samples were ranging from 15 to 34 mmol acid / kg honey. From the table we can see that the lowest acidity shows chestnut honey while the highest acidity shows acacia honey. So all samples have permitted acidity prescribed by regulations on the quality of honey and other bee products.

## CONCLUSIONS

The values of quality parameters for all the honey samples studied coincide with those specified by the international honey regulations.

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