STUDY CONCERNING MICROBIOLOGICAL AND PHYSICAL-CHEMICAL CHARACTERISTICS OF TRANSYLVANIA HONEY

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***Abstract**. The present paper is a comparative microbiological and physical-chemical analysis of various types of honey (polyfloral, tilia, acacia, sunflower, and honeydew) collected from beekeepers and processors. The results have enabled us to make correlations between moisture, acidity, pH and the microbiological characteristics of the tested honey samples.*

Keywords: honey, microbiological analysis, physical-chemical analysis

General aspects

Honey is a sweet and viscous substance produced by the honeybee from the nectar of floral plants. It is produced in almost every country of the world and it is a very important energy food, used as an ingredient in hundreds of manufactured foods, mainly in cereal-based products, for sweetness, flavour, colour, caramelisation and viscosity.
The system of ensuring food products in sufficient amounts is on a continuous descending line. At the present time, the integrity, quality, sanitation and nutritional value of food products are paid increased attention.[1]. The concept of "food safety based on the general hygiene principles of food products and on the HACCP method adopted by Codex Alimentarius comes to reduce or control biological, physical and/or chemical contaminations [2]. Maintaining the contamination level within accessible, minimum limits leads to a linear and constant process when it comes to ensuring quality and food safety. Thus, the harvesting, collecting, manufacturing and storing of honey are a field in a continuous evolution and adjustment to the European legislation, industry needs and the harsh market competition.
Honey represents a semi liquid, yellow, sweet and flavoured foodstuff, with a great biological and caloric value (it contains sugar, vitamins and enzymes), collected and produced by the bees from nectar, manna or sweet juice that can be found in different parts of plants and trees. Being a "noble" product of the bee, honey has a wide range of action depending on its kind [3]. The physical-chemical and hygienic qualities of honey constitute indicators that offer information regarding the energetic and nutritional quality, as well as the possibility of falsifying honey. The authentication of honey is assessed through its physical-chemical parameters falling within the limits imposed by the present legislation. Falsifying agents or inappropriate thermal treatments are identified through the HMF (HydroxyMethylFurfuraldehyde) content.
The microorganisms in honey come from nectar and pollen, from the processing area, from the insufficiently washed machines or containers. The more frequently encountered sporulated microorganisms belong to the Bacillus type. The non-sporulated bacteria (Micrococcus, Pseudomonas, Flavobacterium) are less numerous, coming from the floral organs or the digestive tract of the bees. The filamentous fungi, being more spread in nature and having thermal resistant spores, with a great capacity of surviving, can be introduced in honey even by man, through dust, through the water installations or containers or even by the bees through pollen [4]. The microbes found in honey are not dangerous for the consumers' health. Even if Aspergillus flavus is found, there are no favourable conditions for aflatoxin.
The presence of microorganisms in honey can sometimes influence the stability of the product and its hygienic quality. Normal honey must lack pathogenic microorganisms or microorganisms that produce enteric illnesses.
Microbiological contamination during or after processing honey was demonstrated by the absence of the microorganisms in the samples collected from primary sources and by the presence of a certain type of bacterium (Bacillus spp) and eight types of fungi (more frequently Candida, Aspergillus, Geotrichum and Rhizopus) in the collected samples on local markets. This fact indicates the contamination from secondary sources during manipulations and previous processes. The contamination with fungi and bacteria indicate inadequate hygiene conditions during collecting, manipulating, processing and storing [8].

Experimental

Twenty bulk liquid honey samples of known origin, aseptically collected from beekeepers located in different areas of Transylvania (Romania) during 2008 and presented in table 1, were used for analysis. Each honey sample was purchased in duplicate in sterilised sealed jars of 200 g. The main ***physical and chemical indicators*** (hydroxymethylfurfuraldehyde (HMF), humidity, acidity and pH) that reflect the honey quality were determined according to the methods proposed in the Harmonized Methods of International Honey Commission [9].

According to STAS 784/3-1989, the main physical and chemical characteristics of honey found in Romanian stores are presented in table no. 1.

Table 1. Physical, chemical and microscopic characteristics of honey

Water, % max.	20
Acidity, ml NaOH sol.1N/100g max.	4
Reducing sugar, expressed as invert sugar, %, min.	70
Easily hydrolysable sugar, expressed as sucrose, %, max	5
Diastasic index, min.	6.5
Ash, %, max.	0.5
Specific pollen grains, with reference to the total number of pollen grains examined, %, min.	25
Hydroxymethylfurfuraldehyde (HMF), mg / 100 g max.	1.5 *
Colour index, mm. (Pfund scale)	max.18
Water insoluble substances, max.	0.1
Falsifying agents (artificial invert sugar, industrial glucose or another starch hydrolysing substance, gelatine, gum, cereal flower or other starchy products, artificial colouring bodies, synthetic sweeteners etc.)	0

*Honey found in jars for sale allows for an HMF content of max. 4 mg per 100g.

The HMF content was determined according to the White method using a UV–VIS spectrophotometer (model T80 PG Instruments, UK).

Moisture content was determined measuring the refractive indices at 20^0C by an ABBE refractometer. The corresponding moisture content was calculated from the refractive index of the honey by reference to a standard table.

The acidity of honey is the content of all free acids, expressed in milliequivalents/kg honey.

The honey sample (10 g) was dissolved in 75 ml carbon dioxide-free water and the pH value was measured using a pH-meter (Inolab level 2, WTW). The same solution was titrated with 0.1 M NaOH solution to pH = 8.30, using an automatic titrator (Titroline Alpha Plus, Schott Instruments).

Free acidity, express as milliequivalents or millimoles acid/ kg honey = ml of 0.1 M NaOH x 10.

From a ***microbiological*** point of view, the contamination of the samples was done by determining the total number of aerobic mesophilic bacteria (NTG) and determining the yeasts and moulds. The used diluting liquid was peptonate physiological serum: 10g of sample were homogenised with 90 ml of SFP, obtaining the diluted solution 10^{-1}.

Total number of germs

Petrifilm Aerobic Count Plate is used –3M Microbiology producer USA.

Petrifilm is a reactive film covered with a dehydrated culture medium which contains standard nutrients, a jelly making agent which is soluble in cold water and a tetrazolium indicator which facilitates the enumeration of colonies.

For each sample, two Petrifilms are used, placing them on a flat surface. The upper part of the film is lifted and, with a sterile dropper we put 1 ml at a time from the diluted solution 10^{-1} on each of the two slates, the upper film is placed on the sample and is distributed with the help of the applying tool, and then some time is given to let it solidify for at least one minute. The Petrifilms are incubated at 30°C ± 1ºC for 72h. All the red colonies are counted, regardless of the size or intensity.

Yeast and mould]

Take two sterile boxes of Petri. With a sterile dropper pour 1 ml of the 10^{-1} diluted solution in every box. About 15 ml of yeast-glucose-cloramfenicol-agar extract is poured (Orgenics producer), previously melted and maintained at $45^0\pm1^0$C in water, in every Petri box. Everything is carefully mixed, it is left to solidify, placing the Petri boxes on a horizontal, cold surface. A witness box is prepared, with 15 ml medium, in order to verify the sterility. The boxes are placed with the lid down, in the incubator, $25^0\pm1^0$C. After three, four and five days of incubation, the colonies from each Petri box are counted. Evaluation: 100 CFU/g.

In order to identify the species and capture the images, a Hund Wetzlar H600LL microscope connected to a PC was used, using the Pinuacle TV Centre programme.

Results and discussion

Table 1 shows the physical-chemical indices: moisture content, pH, acidity and HMF of analyzed samples.

Table 1. The results of the physical-chemical analyses of the honey samples

Sample no.	Sample code	Moisture content (%)	pH	Acidity (meq /kg)	HMF (mg/kg)
1.	Polyfloral honey	15.6	3.72	41	12.2
2.	Polyfloral honey	16.8	3.99	23	***52.3***
3.	Linden honey	16.4	4.39	16	14.9
4.	Linden honey	17	4.86	11	1.0
5.	Acacia honey	18	3.75	12	4.3
6.	Acacia honey	19.4	2.89	14	8.4
7.	Forest honey	16.2	3.66	28.9	5.7
8.	Forest honey	15	3.98	43.8	21.7
9.	Sun flower honey	16.4	3.67	22.6	22.3
10.	Sun flower honey	19.8	3.59	20.9	23.8
11.	Polyfloral honey	16.4	3.6	37.3	31.8
12.	Polyfloral honey	15.4	4.01	20.6	7.4
13.	Polyfloral honey	15.6	3.9	***55.6***	4.5
14.	Polyfloral honey	16.4	3.78	28.5	2.3
15.	Polyfloral honey	16.6	4.04	30.4	8.5
16.	Polyfloral honey	14.6	3.8	24.4	30.0
17.	Linden honey	16.4	4.36	20.0	24.8
18.	Linden honey	16.4	4.01	23.4	14.2
19.	Acacia honey	16.4	3.9	14.3	***64.3***
20.	Acacia honey	16.8	3.9	14	10.7

The physical-chemical parameters values were within the reference ranges presented in UE or national reglementation. Exception is sample number 2 and 19 for HMF value and sample 13 for acidity level. In order to establish the microbiological characteristics of the honey samples, the parameters from table 2 were analysed.

Table 2. The microbiological control of the honey samples

Number of the sample	NTG/g	DM/g
1	30	30 (*Penicillium* spp.)
2	95	15 (*Rhizopus* spp., *Aspergillus* spp., *Penicillium* spp.)
3	45	<10
4	15	15 (*Absidia* spp.)
5	20	<10
6	20	20 (*Penicillium* spp.)
7	40	40 (*Penicillium* spp., *Aspergillus* spp.)
8	45	20 (*Penicillium* spp., *Aspergillus* spp.)
9	35	<10
10	10	10 (*Fusarium* spp.)
11	30	<10
12	25	<10
13	<10	<10
14	20	10 *Aspergillus* spp
15	45	10 (*Penicillium* spp.)
16	10	<10
17	20	<10
18	25	<10
19	<10	<10
20	20	<10

The antimicrobial character of honey is confirmed by the results regarding the TNG (the total number of germs): in all the analysed samples it is under 100 CFU/g – value settled by the present legislation- which represents a reduced contamination with aerobic mesophilic germs. Out of the 20 analysed samples, the greatest microbiological importance was represented by sample no. 2 with 95 CFU/g.

Regarding the yeasts and moulds, we notice that there is no yeast in all the 10 analysed samples and the number of mould does not exceed 40/g in any sample. The most frequently encountered one is the *Penicillium* type (in five samples), then *Aspergillus* (in three samples). In each sample the fungi from the *Absidia (Mycocladus), Rhizpus* and *Fusarium* types were determined. We can notice that in the polyfloral honey sample fungi from the *Penicillium, Aspergillus* and *Rhizopus* types can be found. Also, fungi of the *Penicillium* type were identified in the acacia honey sample.

We can notice the presence of TNG in the samples subjected to various handling and also a variety regarding the types of fungi encountered in the honey samples. This fact confirms a contamination from a microbiological point of view during its manipulation by the beekeepers and the primary honey treatments, which indicate unsatisfying hygiene conditions.

Figures 6 and 7 present the results of the correlated log of bacterial number and moisture separately for polyfloral honey samples from other samples and except the two samples (13 and 19), where the microbial growth is lower than 10.

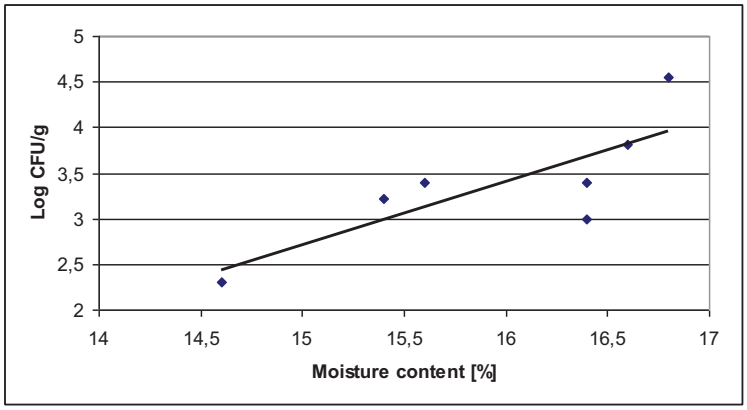

Fig. 6. Correlation between total counts in polyfloral honey sample and their moisture content

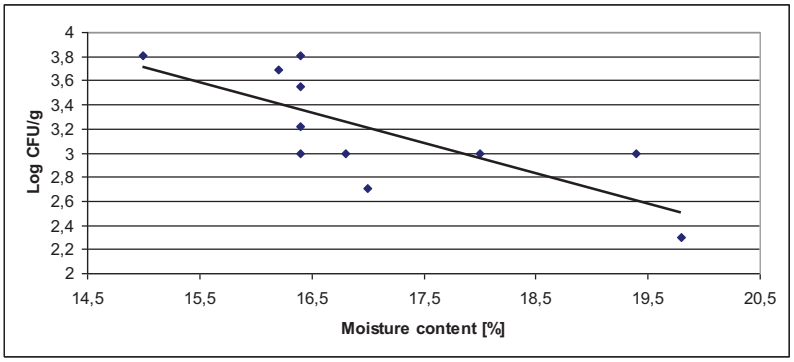

Fig. 7. Correlation between total counts in linden, acacia, forest and sun flower honey sample and their moisture content

Slope of regression line log of bacterial number over moisture is y = 0,6976x - 7,7593, r^2 = 0,6378 in case of polyfloral honey samples and y = -0,253x + 7,5075, r^2 = 0,5714 for linden, acacia, forest and sun flower honey sample.

The dependences between TNG and the water content from the analysed samples were emphasized. The correlative analysis shows that there are appreciating correlations between the microbiota and the physical-chemical honey parameters.

Conclusions

The study allowed the qualitative analysis of the honey samples collected from beekeepers in Transylvania.

The experimental values of the physical-chemical and microbiological parameters of honey demonstrate the following:

The presence of mould (especially of mould) in the *Penicillium, Aspergillus, Absidia, Rhizpus, Fusarium* types, but which cannot exceed the limit values. These facts, as well as the favourable conditions can lead to generating and developing micotoxins.

Contamination from secondary sources during the manipulations due to the inadequate hygiene conditions during the selection, manipulation and storing.

The importance of corresponding processing of honey: filtration, dehydration, liquefaction, pasteurization (70-78^{0}C for 5-6 min.), cooling (sudden at 42^{0}C) and wrapping in order to stop or destroy the present microorganisms.
The physical-chemical parameters were within the limits imposed by the present legislation, except for 2.13 and 19 samples.
Correlating the physical-chemical and microbiological results is necessary in order to check the quality and the sanitation of honey. This fact constitutes practical proof in ensuring food safety.

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