



## MICROBIOLOGICAL AND PHYSICO – CHEMICAL QUALITY OF HONEY COLLECTED FROM DIFFERENT SLOVAK HABITATS

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### ABSTRACT

This study was intended to determine microbiological quality and physiochemical characteristics of honey collected from different Slovak habitats. Physiochemical properties were determined using the harmonised methods of the international honey commission. We found no occurrence of coliform bacteria in honey through microbiological analysis. Number of microscopic fungi was in amount from 100 to 4500 cfu.g-1. From isolated species composition of fungi the most frequent genera included *Alternaria* sp., *Mycelia sterilia* and *Aspergillus candidus*. All samples of honey were well within the limits of the Codex Standard for water content, conductivity, diastase, invertase, pH and water activity.

**Key words:** microbiological quality, physiochemical characteristics, coliform bacteria, microscopic fungi, honey

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### INTRODUCTION

Honey, the nectar and sweet deposits from plants which are gathered, modified and stored in the honeycomb by honeybees, is a popular sweetener. New technologies and innovative uses of honey are expanding marketing opportunities. However, new microbiological requirements related to quality and safety may be associated with these opportunities. A more comprehensive understanding of honey's microbiological characteristics is needed as honey is used in new ways (Snowdon and Cliver, 1996).

The following information leads into the conclusion that primary sources of microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust,

air, dirt and flowers. Secondary sources of microbes in honey are likely to be the same as for other foods.

Many microorganisms are associated with specific foods or components of the ecosystem (Jay, 2000). Organisms found in the environment around honey (i.e. bees, hives, pollen, flowers, soil etc.) are also likely to occur in honey. *Actinetobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Pseudomonas*, *Psychrobacter* and *Vagococcus* are bacteria commonly found in soil. Air and dust are important sources of *Bacillus*, *Clostridium* and *Micrococcus species*. *Bacillus* and *Clostridium* species are important bacterial contaminants of cane and beet sugars, *Saccharomyces* and *Torula* yeasts can be found in high-moisture sugars, and *Leuconostoc mesenteroides* has been found in sugar refineries. *Brochothrix*, *Citrobacter*,

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*Enterobacter*, *Erwinia*, *Flavobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria* and *Pediococcus* are found in plants and plant products.

Honey is a supersaturated solution of saccharides and the mean value of water activity for honey ranges from 0.562 to 0.620 (Tysset et al., 1980). With the exception of osmotic kinds of yeasts, this environment is unsuitable for the survival of microorganisms (Molan, 1992). Some authors claim that the cause of the antimicrobial effect of honey solution is its high osmolarity, thus it is not different from a normal solution of saccharides and the antimicrobial effect results from the physical and not the chemical character of honey (Grobler and Basson, 1996). However, the results of many a studies show that many factors contribute to the antimicrobial character of honey (Molan, 1992). Sugar solutions and pastes have a high osmolarity but they are therapeutically inefficient. The low pH of honey, ranging from 3.2 to 4.5 further explains its antimicrobial activity. Acidity is primarily determined by the content of gluconic acid, which results from the enzyme reaction while nectar is ripening (Molan, 1992).

## MATERIAL AND METHODS

### Microorganisms in honey

About 30 samples collected from commercial lots of honey in various areas of Slovakia were used for analyses.

### Determination of cfu counts

Plate diluting method was applied for quantitative cfu counts of respective groups of microorganisms in 1 g of honey. Gelatinous nutritive substrate in petri dishes was inoculated with 1 ml of honey samples by flushing and on surface in three replications.

### Dilution of the samples

Basic dilution ( $10^{-2}$ ) was prepared as follows: 5 g of honey content was added to the test tube containing 45 ml of distilled water.

**Table 1: Applied nutritive substrates**

| Microbial groups | Medium                             | Length of incubations (h) | Cultivation method | Temperature (°C) |
|------------------|------------------------------------|---------------------------|--------------------|------------------|
| Coliforms        | Violet red bile agar               | 24                        | aerobic            | 37               |
| Moulds           | Czapek<br>- Dox agar,<br>Malt agar | 120                       | aerobic            | 25               |

## Physical and chemical parameters

The determination of physical and chemical parameters such as content of water, conductivity, pH and invertase and diastase activities was carried out in accordance with the methods described in Harmonized methods of the European Honey Commission (Bogdanov et al., 1997).

### pH determination

The pH value was determined in a solution containing 10 g of honey in 75 ml of distilled water free of CO<sub>2</sub>. Each sample was analysed in three parallel determinations.

### Water activity (*a<sub>w</sub>*)

Water activity was determined with the help of *a<sub>w</sub>*-meter. Each sample was analysed in three parallel determinations.

## RESULTS AND DISCUSSION

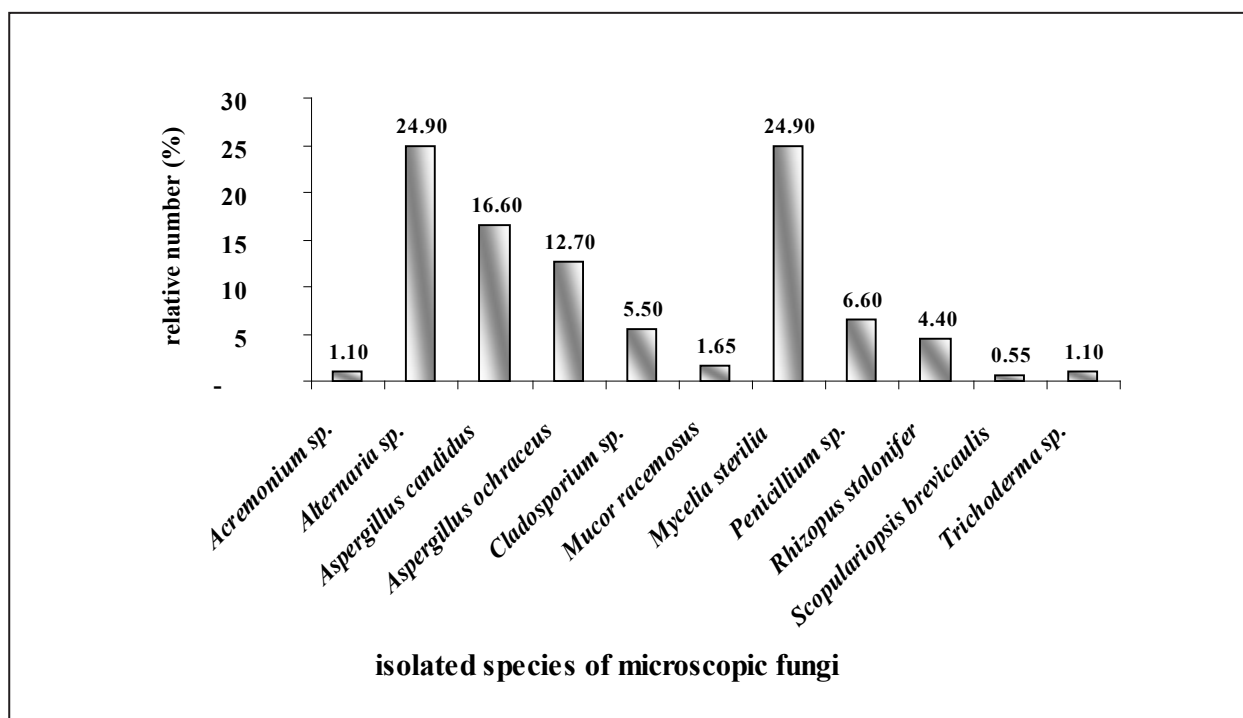
### Microbiological characteristics of honey samples

Results of our experiments showed the presence of microscopic fungi in amount from 100 to 4500 cfu.g<sup>-1</sup> among monitored groups of microorganisms in 30 honey samples. From isolated species composition amongst fungi the most frequent genera included *Alternaria sp.*, *Mycelia sterilia* and *Aspergillus candidus*. We did not find any coliform bacteria in the honey samples from different Slovak habitats.

Knowledge of the moisture and temperature conditions influencing growth of microorganisms in honey was used as control of the spoilage of honey for long time. However, the requirement for additional microbiological data on honey will increase as new technologies for, and uses of honey develop. Microorganisms in honey may influence quality or safety. Due to the natural properties of honey and control measures in the honey industry, honey is produced with minimal types and levels of microbes. Microbes of concern in post-harvest handling are those that are commonly found in honey (i.e., yeast and spore-forming bacteria), those that indicate the sanitary or commercial quality of honey (i.e., coliforms and yeasts), and those that under certain conditions could be the reason of human illness.

**Table 2: Absolute and relative number of isolated species of microscopic fungi in honey samples**

| Isolated species of microscopic fungi | Absolute number | *Relative number (%) |
|---------------------------------------|-----------------|----------------------|
| <i>Acremonium sp.</i>                 | 200             | 1.10                 |
| <i>Alternaria sp.</i>                 | 4500            | 24.90                |
| <i>Aspergillus candidus</i>           | 3000            | 16.60                |
| <i>Aspergillus ochraceus</i>          | 2300            | 12.70                |
| <i>Cladosporium sp.</i>               | 1000            | 5.50                 |
| <i>Mucor racemosus</i>                | 300             | 1.65                 |
| <i>Mycelia sterilia</i>               | 4500            | 24.90                |
| <i>Penicillium sp.</i>                | 1200            | 6.60                 |
| <i>Rhizopus stolonifer</i>            | 800             | 4.40                 |
| <i>Scopulariopsis brevicaulis</i>     | 100             | 0.55                 |
| <i>Trichoderma sp.</i>                | 200             | 1.10                 |
|                                       | 18100           | 100.00               |

**Fig. 1: Relative number of isolated species of microscopic fungi in honey samples**

Primary sources of microbial contamination probably include the pollen, the digestive tracts of honeybees, dust, air, earth and nectar - sources that are very difficult to control. The same secondary (post-harvest) sources that influence other food products are

also sources of contamination for honey. These include air, food handlers, cross-contamination, equipment and buildings. Secondary sources of contamination are controlled by good manufacturing practices (Kačániová, 2005a).

The microbes of concern in honey are primarily yeast and spore-forming bacteria. Total plate counts from honey samples can vary from zero to tens of thousands per gram for no apparent reason. Most samples of honey contain detectable levels of yeasts. Although yeast counts in many honey samples are below 100 colony forming units per gram ( $\text{cfu.g}^{-1}$ ), yeasts can grow in honey in very high numbers. Standard industry practices control yeast growth. Bacterial spores, particularly those belonging to the *Bacillus* genus, are regularly found in honey. The spores of *C. botulinum* are found in a fraction of the honey samples tested—normally at low levels. We found no vegetative forms of disease-causing bacterial species in honey. Bacteria do not replicate in honey and as such high numbers of vegetative bacteria could indicate recent contamination from a secondary source. Certain vegetative microbes can survive in honey, at cool temperatures, for several years. However, honey has anti-microbial properties that discourage the growth or persistence of many microorganisms. Typically, honey can be expected to contain low numbers and a limited variety of microbes (Kačániová et al., 2004).

A routine microbiological examination of honey might include several different assays. A standard plate count provides general information. Specialized tests, such as a count of yeasts and an assay for bacterial spore-formers, may also be useful. An indicator of sanitary quality as provided by coliform counts might be included. Additional tests, to explain unusually high counts or address a certain problem, may be needed.

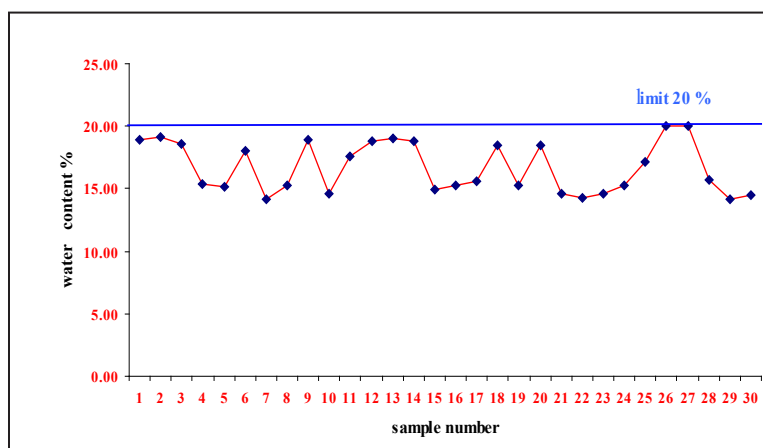


Fig. 2: Water content in % of honey

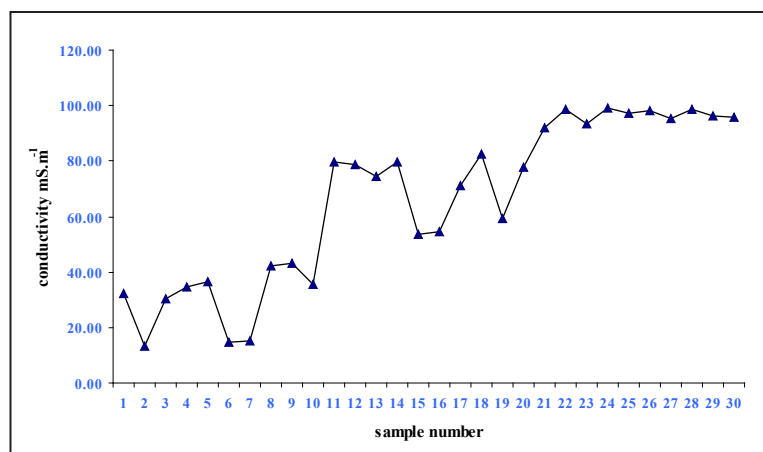
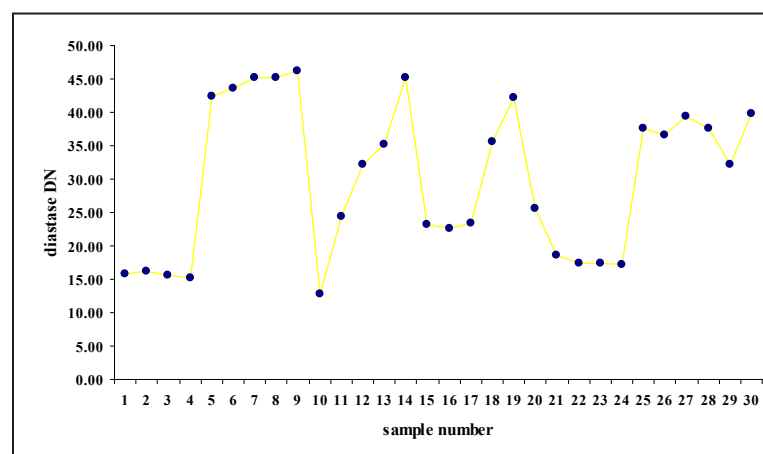
Fig. 3: Conductivity in  $\text{mS.m}^{-1}$  of honey

Fig. 4: Diastase in diastatic numbers in honey

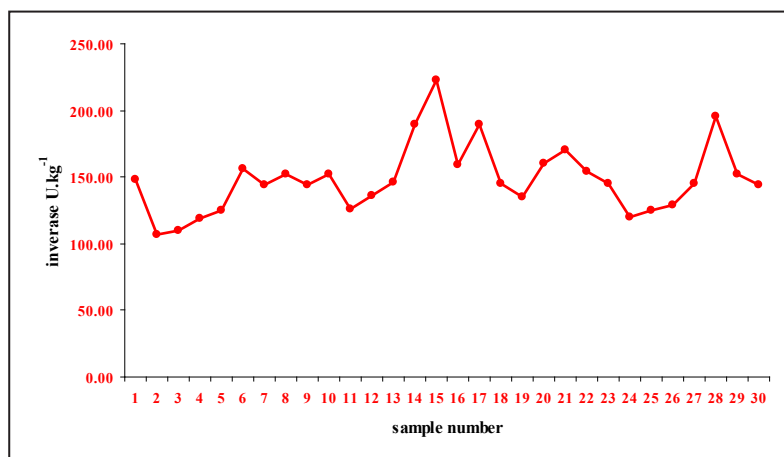


Fig. 5: Invertase in U.kg<sup>-1</sup> of honey

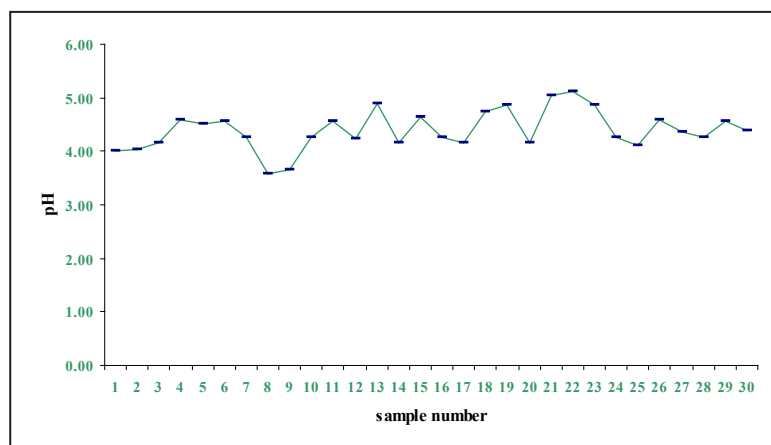


Fig. 6: pH of honey

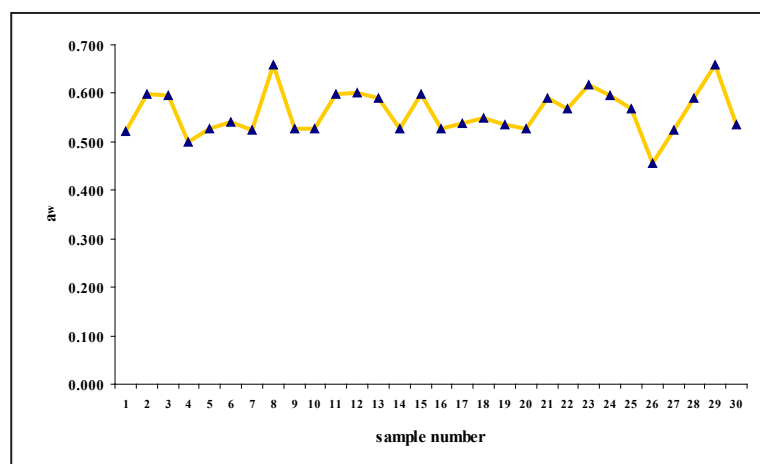


Fig. 7: Water activity of honey

The use of honey in products that receive no or limited heat treatment may require additional tests. More information on the source and control of microbes in honey is needed to answer the concerns currently facing the industry (Kačániová, 2005b).

### Physicochemical parameters of honey

The results of the determination of physicochemical parameters of honey for individual samples are shown in Figure 2. -7.

Table 3 summarizes the basic statistical evaluation of honey divided into three groups. The observation of the water activity did not show any significant difference between the types of honeys. The found values ranged between 0.460 (14.14 % of water) and 0.660 (20.00 % of water). The increased water activity influences the shelf life of honey and supports the growth of undesirable microflora, especially osmotolerant yeasts. The highest value of *aw* (0.660) in samples analyzed in this study was established in honeydew type of honey. However, this value is safe from the aspect of possible growth of microorganisms. The statistical significance of measured pH values was variable in different groups of honey. The highest values were found in the group of honeydew honey (5.12) and the lowest in blossom honey (3.58). It is in accordance with bibliographic references that also state that the pH value is influenced by organic acids and by the concentration of mineral substances (Crane, 1990). The values of the activity of the two most important enzymes diastase and invertase, which are used in some countries as the legislative criteria of honey quality (Bogdanov et al., 1997), proved differences between blossom and honeydew honeys, which are in accordance with several other reports (Krauze and Zalewski, 1991; Vorlová and Přidal, 2002). No differences were proved between blends and blossom honeys. Similar results in respect of physicochemical characteristics of honey samples were also reported by Vorlová et al. (2005).

**Tab. 3: Statistical evaluation of physicochemical parameters of honey**

| <sup>1</sup> Type of honey    |           | <sup>5</sup> Water content<br>% | <sup>6</sup> Conductivity<br>mS.m <sup>-1</sup> | <sup>7</sup> Diastase<br>DN | <sup>8</sup> Invertase<br>U.kg <sup>-1</sup> | pH    | a <sub>w</sub> |
|-------------------------------|-----------|---------------------------------|---|-----------------------------|--|-------|----------------|
| <sup>2</sup> Blossom<br>n=10  | $\bar{x}$ | 16.81                           | 29.810  | 29.810                      | 135.760                                      | 4.160 | 0.550          |
|                               | SD        | 2.06                            | 11.320  | 15.550                      | 18.700                                       | 0.350 | 0.050          |
|                               | min       | 14.16                           | 13.450  | 12.850                      | 106.800                                      | 3.580 | 0.500          |
|                               | max       | 19.10                           | 43.250  | 46.120                      | 156.120                                      | 4.580 | 0.660          |
| <sup>3</sup> Blends<br>n=10   | $\bar{x}$ | 17.24                           | 71.130  | 30.960                      | 160.840                                      | 4.470 | 0.560          |
|                               | SD        | 1.76                            | 11.060  | 8.340                       | 30.720                                       | 0.310 | 0.030          |
|                               | min       | 14.89                           | 53.550  | 22.660                      | 125.690                                      | 4.150 | 0.530          |
|                               | max       | 19.07                           | 82.500  | 45.210                      | 223.120                                      | 4.890 | 0.600          |
| <sup>4</sup> Honeydew<br>n=10 | $\bar{x}$ | 16.34                           | 96.500  | 29.340                      | 148.120                                      | 4.560 | 0.570          |
|                               | SD        | 3.01                            | 2.330   | 10.320                      | 22.360                                       | 0.350 | 0.060          |
|                               | min       | 14.14                           | 92.190  | 17.250                      | 120.000                                      | 4.120 | 0.460          |
|                               | max       | 20.00                           | 99.120  | 39.850                      | 195.610                                      | 5.120 | 0.660          |

$\bar{x}$  = average, SD = standard deviation, n = numer of samples, a<sub>w</sub> = water activity

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