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QUALITY EVALUATION OF UNIFLORAL AND MULTIFLORAL HONEYS FROM SLOVAKIA AND OTHER COUNTRIES

Vladimíra Kňazovická[1], Bianka Mederiová[2], Peter Haščík[1], Miroslava Trnková[2], Miroslava Kačániová[3]*

Address(es): Ing. Vladimíra Kňazovická, PhD.
[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Products Evaluation and Processing, Tr. A. Hlinku 2, 949 76 Nitra, The Slovak republic, phone number: +421 37 641 4428.
[2]Graduated in Food Technology (in 2012) at Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra.
[3]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, The Slovak republic.

*Corresponding author:vladimira.knazovicka@uniag.sk

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ABSTRACT

The aim of the study was to evaluate the blossom honey samples divided into the three groups: acacia (*Robinia psedoacacia*) honeys (n=14), other unifloral honeys (clover *Trifolium pratense*, lime *Tilia cordata*, rape *Brassica napus*, buckwheat *Fagopyrum esculentum*, chestnut *Castanea sativa*; n=8) and multifloral honeys (n=16). We tested the physico-chemical and microbiological quality of honeys. Followed physico-chemical parameters were tested: water, water content, hydroxymethylfurfural (HMF), pH, free acidity and diastase. From microbiological parameters, we found total plate count (TPC), TPC of anaerobic microorganisms, counts of coliform bacteria, sporulating bacteria and microscopic fungi in the honey samples using dilution plating method. Water content ranged from 13.60 to 21.90 g $100g^{-1}$ and two samples of multifloral honeys exceeded the limit value for water content (max 20.00 g $100g^{-1}$). HMF ranged from 0.77 to 10.93 mg kg^{-1} that is typical for fresh and heat-untreated honeys. Average values of pH were 5.02 ± 0.25 for acacia honey, 4.98 ± 0.31 for other unifloral honeys and 4.66 ± 0.45 for multifloral honeys. One sample of multifloral honeys exceeded the limit value for free acidity (max 50 meq kg^{-1}). Higher TPC (above 2.00 log CFU g^{-1}) was detected in 2 out of 14 acacia honeys (14.29%), in 2 out of 8 other unifloral honeys (25.00%) and in 5 out of 16 multifloral honeys (31.25%). Microscopic fungi were not found in 3 acacia honeys (21.43%), 2 other unifloral honeys (25.00%) and 2 multifloral honeys (12.50%). Overall, the obtained results showed good quality of evaluated honeys.

Keywords: Blossom honey, physico-chemical parameters, microorganisms

INTRODUCTION

Blossom honey and honeydew honey are two main honey types in term of its origin. Blossom honey comes from nectar of plants. In practical terms there are unifloral and multifloral types of blossom honey. Beekeepers only obtain unifloral honey from profuse crops, for example *Brassica napus*, *Robinia pseudoacacia* or *Rubus ideaus* (**Veselý *et al.*, 2003**). In such a case, the honey is labelled according to the dominant plant species (**Kamler *et al.*, 1999**). Multifloral honey is accrued from several plant sources; bees deposit nectar and pollen of several plants and transfer from cell to cell depending on necessity (**Titěra, 2006**). The use of botanical appellation of honey together with geographical origin is becoming a good option to protect and promote this traditional food in different countries (**Juan-Borrás *et al.*, 2014**). It is expected that honey properties from different botanical sources and location are different (**Mărghitas *et al.*, 2009**). In Slovakia typical unifloral honeys are from lime (*Tilia cordata*), acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annus*), rape (*Brassica napus*) or clover (*Trifolium pratense*).

Honey consists of sugars, water, organic acids, enzymes and other substances as proteins, aminoacids, vitamins, minerals, acetylcholine, flavonoids and various organic compounds (pollen grains, wax particles) (**Kukurová *et al.*, 2009**). In general blossom honeys contain higher amount of proteins, smaller amount of minerals and oligosaccharides compared to honeydew honeys. Main sugars in blossom honeys are the monosaccharides fructose and glucose and the principal oligosaccharides are the disaccharides sucrose, maltose, trehalose and turanose as well as some nutritionally relevant ones such as panose, 1-kestose, 6-kestose and palatinose (**Bogdanov *et al.*, 2008**). Characteristic compound of honey, produced from the acid-catalyzed degradation of sugars (mainly fructose), is HMF (5-hydroxymethylfurfural) (**Zielińska *et al.*, 2014**), which appears during ageing or heating of honey (**Amir *et al.*, 2010**). The water content is a good criterion to establish the quality of honey; a higher content can produce honey fermentation during storage (**Mărghitas *et al.*, 2009**). Enzymes are the most important and also the most interesting honey components; they are accountable for the conversion of nectar to honey, and serve as a sensitive indicator of the honey treatment (**Vorlová and Čelechovská, 2002**).

One of the honey valuable properties is its antimicrobial activity against pathogenic vegetative bacteria, microscopic filamentous fungi and protozoa **(Kisała and Dżugan, 2009)**. The natural acidity of this product, the low protein content and the high viscosity, that limit the atmospheric oxygen penetration, are particularly stressing for several microorganisms (**Sinacori *et al.*, 2014**). However, honey is not sterile material completely. The predominant microorganisms found in honey are derived from the nectar and the honey bee (**Chaven, 2014**). The biodiversity and quantity of microorganisms in honey are variable and unique to the honey sample analysed. They are dependent on honey quality (properties of honey), which is determined by a variety of factors during honey production and treatment. According to **Snowdon and Cliver (1996)** microbial survival may be influenced mainly by the type of honey and its moisture content. **Sinacori *et al.* (2014)** analyzed some nectar (blossom) and honeydew honeys from Italia and they found 13 species of bacteria, 5 species of yeasts and 17 species of microscopic filamentous fungi; the species most frequently isolated were *Bacillus amyloliquefaciens*, *Zygosaccharomyces mellis* and *Aspergillus niger* for the three microbial groups, respectively. Sporulating bacteria, microscopic filamentous fungi and yeasts are typically found in honey, often at low numbers, while spores can persist indefinitely **(Snowdon and Cliver, 1996)**. According to **Różańska (2011)** bacteria are not able to multiply in honey; their high number could indicate contamination during processing, handling or storage. Vegetative forms of pathogenic bacteria have never been found in honey (**Snowdon and Cliver, 1996**).

The aim of the study was to evaluate three groups of blossom honey samples: acacia honeys, other unifloral honeys and multifloral honeys. Samples were analyzed from physico-chemical and microbiological viewpoint.

MATERIAL AND METHODS

Honey samples

We analysed 38 samples of blossom honeys. Honeys originated mainly in Slovakia (n=35) and they were divided into three groups: acacia honeys (n=14), other unifloral honeys (n=8): clover (*Trifolium pratense*, n=1), lime (*Tilia cordata*, n=1), rape (*Brassica napus*, n=3), buckwheat (*Fagopyrum esculentum*, n=1), chestnut (*Castanea sativa*, n=2) and multifloral honeys (n=16). The detailed characterization of evaluated samples is in the Table 1. Honeys were produced in 2009-2010. Before the physico-chemical parameters were determined, the primary treatment of samples in accordance with the **IHC (2009)** was performed, which emphasizes sample homogenization and the exclusion of air.

Physico-chemical analyses

The water content and water activity were measured simultaneously. The water content was detected using a portable refractometer HHR-2N (ATAGO®, Japan) and water activity was detected using a Novasina LabMaster (Pfaffinon, Switzerland).
HMF content was measured by using the RQflex 10® and Hydroxymethylfurfural test (Merck, Germany) in undiluted honey samples.
The free acidity was measured by titration method according to the **IHC (2009)**. And pH of honey solution intended for free acidity detection was measured using Whatman® pH indicator papers with range of pH 3.8-5.5 (Whatman®, UK).
Diastase activity was detected according to Phadebas (Phadebas®, Magle AB, Sweden) in accordance with **IHC (2009)**.

Microbiological analyses

The samples were analyzed by dilution plating method for the quantitative determination of total plate count (TPC), TPC of anaerobic microorganisms, counts of coliform bacteria, sporulating bacteria and microscopic fungi (yeasts and microscopic filamentous fungi). The characteristics of method are shown in the Table 2.

Table 1 Characterisation of blossom samples

no.	Honey type	Geographical location	Production year
1	acacia	Drazovce-Nitra, SR	2009
2	acacia	Hanusovce nad Toplou, SR	2009
3	acacia	Velky Meder, SR	2009
4	acacia	Krupina, SR	2009
5	acacia	Bzovik, SR	2009
6	acacia	Piestany, SR	2009
7	acacia	Vranov nad Toplou, SR	2009
8	acacia	Trnovec nad Vahom, SR	2009
9	acacia	Sala, SR	2009
10	acacia	Sebechleby, SR	2009
11	acacia	SR	2009
12	acacia	Rovne - Humenne, SR	2009
13	acacia	Kochanovce - Humenne, SR	2009
14	acacia	SR	2009
15	buckwheat	Nedanicky, CR	2009
16	clover	Voderady, SR	2009
17	lime	Sebechleby, SR	2009
18	rape	Sebechleby, SR	2009
19	rape	Michalovce, SR	2009
20	rape	Rovne - Humenne, SR	2009
21	chestnut	Menges, Slovenia	2010
22	chestnut	V. Kladusa, Croatia	2010
23	multifloral	Luckovce, SR	2009
24	multifloral	Detva, SR	2009
25	multifloral	Filakovo, SR	2009
26	multifloral	Kosice, SR	2009
27	multifloral	Kysak, SR	2009
28	multifloral	Stupava, SR	2009
29	multifloral	Bojnice, SR	2009
30	multifloral	Lefantovce, SR	2009
31	multifloral	Lefantovce, SR	2009
32	multifloral	Trnavoc nad Vahom, SR	2009
33	multifloral	Sala, SR	2009
34	multifloral	Velky Lapas, SR	2009
35	multifloral	Trebisov, SR	2009
36	multifloral	Male Krstenany, SR	2010
37	multifloral	SR	2010
38	multifloral	SR	2010

Legend: clover (*Trifolium pratense*), lime (*Tilia cordata*), rape (*Brassica napus*), buckwheat (*Fagopyrum esculentum*), chestnut (*Castanea sativa*), SR – The Slovak republic, CR – The Czech republic

Statistical analysis

The measurement data were calculated to appropriate units. For each honey group, followed statistical parameters were found by MS Excel 2007: the number of values with measured (non-zero) values (n), average, standard deviation (SD), minimum (min), maximum (max) and coefficient of variation (coef. var.).

Table 2 Microbiological analysis of honey

Microbial group	Medium	Inoculation	Conditions of cultivation		
			O_2 Req.	Temp.	Time
Coliform bacteria	VRBL	spreading	aerobic	37 °C	24 h
TPC	GTY	pouring	aerobic	30 °C	48-72 h
TPCan	NA 2	pouring	anaerobic	25 °C	48-72 h
Sporulating bacteria	AA	pouring	aerobic	37 °C	48-72 h
Microscopic fungi	CD, MA	pouring	aerobic	25 °C	5-7 days

Legend: Req. – requirement, Temp. – temperature, TPC – total plate count, TPCan – total plate count of anaerobic microorganisms, VRBL – violet red bile lactose agar, GTY – agar with glucose, tryptone and yeast extract, NA 2 – nutrient agar no. 2 (Imuna); AA – anaerobic agar (particularly for *Clostridium* sp.), MA – malt agar (Biomark Laboratories); CD – Czapek-Dox agar (Oxoid)

RESULTS AND DISCUSSION

Physico-chemical quality of honeys

The results from the physico-chemical analyses of acacia, other unifloral and multifloral honeys are recorded in the Table 3, 4 and 5.

The water content is a quality parameter, important above all for honey shelf life (**Bogdanov *et al.*, 2004**). Honey contains 15-21% of water (**Kukurová *et al.*, 2009**). We found water content from 13.30 to 21.90 g $100g^{-1}$ in analyzed blossom samples. Three samples exceeded the limit value of **Codex Stan (2001)** that is 20 g $100g^{-1}$. Water content was detected from 15.7 to 21.7 g $100g^{-1}$ in Polish blossom honeys (**Zielińska *et al.*, 2014**) and from 15.40 to 20 g $100g^{-1}$ in Romanian blossom honey (**Mărghitas *et al.*, 2009**). According to **Chaven (2014)**, honey with 1000 yeast spores or less per gram will remain stable at water content between 17.1% and 18.0%, while if the water content is between 18.1% and 19.0%, it would be expected that yeast would be able to grow and ferment the honey.

Water activity of tested honeys ranged from 0.485 (in acacia honey) to 0.629 (in multifloral honey). Most honeys possess a water activity of approximately 0.600, and many microbial species require water activity values between 0.940 and 0.990 in order to grow (**Cooper, 2005**). Yeasts require lower minimal water activity, between 0.910 and 0.880 and osmotolerant species *Zygosaccharomyces rouxii* and *Z. bailii* are capable of reproducing when the water activity is as low as 0.73 (**Šilhánková, 2002).**

HMF values in analyzed samples ranged from 0.77 to 10.93 mg kg^{-1}. All samples were in accordance with **Codex Stan (2001)**, where the limit value is 40 mg kg^{-1}. **Zielińska *et al.* (2014)** found HMF in concentration from 0.5 to 28.0 mg kg^{-1} in the Polish unifloral and multifloral honeys. **Amir *et al.* (2010)** found very low HMF content in fresh Algerian honeys, HMF values ranged from 0.003 to 1.29 mg kg^{-1}. HMF level serves as a quality measure for excessive heat during extraction, storage changes as HMF increases over time, or for possible adulteration with sugars and syrup (**Chaven, 2014**). Heat-treatment of honey causes the degradation of vitamins, nutritional components and decreases the diastase activity and increases the HMF content (**Kukurová *et al.*, 2009**).

The average value of free acidity was 12.31 ± 2.69 meq kg^{-1} in acacia honey, 20.50 ± 9.30 meq kg^{-1} in other unifloral honeys and 24.27 ± 13.24 meq kg^{-1} in multifloral honeys. One sample exceeded the limit value of **Codex Stan (2001)** that is max 50 meq kg^{-1}. **Zielińska *et al.* (2014)** found similar free acidity in unifloral and multifloral Polish honeys (from 9.0 to 50.8 meq kg^{-1}) and they found the highest acidity in the buckwheat honey, while the rape honey was characterized by the lowest content of this parameter. The pH values in the analyzed samples ranged from 4.60 to 5.50 in acacia and other unifloral honeys and from 3.80 to 5.20 in multifloral honeys. According to **Bogdanov *et al.* (2004)** all honeys are acidic with pH-value of unifloral honeys generally lying between 3.5 and 5.5, due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage. **Kisala and Džugan (2009)** stated that one of the physico-chemical factors influenced the antimicrobial activity is acidity of honey (with the average pH value 4.1), which is caused by the organic acids presence; acidity together with high osmotic pressure hinder the development of bacteria and osmophile yeasts.

Honey contains small amounts of different enzymes, the most important ones being diastase (α- and β-amylase), invertase (α-glucosidase), glucose oxidase, catalase and acid phosphatase, which come from nectar sources, salivary fluids and from the pharyngeal gland secretions of the honeybee (**Serrano *et al.*, 2007**). We detected the diastase number, which ranged from 10.45 DN (in acacia honey) to 52.36 DN (in multifloral honey). The lowest diastase average value was found in acacia honeys (18.60 ± 7.19 DN). Similar results for acacia honey from Spain published **Juan-Borrás *et al.* (2014)**. The highest average value was found in multifloral honeys (25.81 ± 10.38 DN). All samples were in accordance with **Codex Stan (2001)**. According to **Kukurová *et al.* (2009),** enzyme activity decreases at 25 °C after 8-10 months of storage.

Table 3 Physico-chemical quality of acacia honeys

	Water content [g $100g^{-1}$]	Water activity	HMF [mg kg^{-1}]	pH	Free acidity [meq kg^{-1}]	Diastase [DN]
n	14	14	11	14	14	14
average	15.78	0.529	3.18	5.02	12.31	18.60
SD	1.47	0.030	1.37	0.25	2.69	7.19
min	13.60	0.485	0.97	4.60	8.90	10.71
max	19.00	0.586	5.59	5.50	18.00	35.38
coef. var. [%]	9.30	5.72	43.10	4.95	21.88	38.67

Legend: HMF – hydroxymethylfurfural, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Table 4 Physico-chemical quality of other unifloral honeys

	Water content [g $100g^{-1}$]	Water activity	HMF [mg kg^{-1}]	pH	Free acidity [meq kg^{-1}]	Diastase [DN]
n	8	8	7	8	8	8
average	16.74	0.548	5.00	4.98	20.50	23.85
SD	1.37	0.030	2.94	0.31	9.30	7.58
min	14.80	0.507	2.20	4.60	12.50	10.45
max	18.30	0.585	10.93	5.50	41.50	31.63
coef. var. [%]	8.18	5.53	58.82	6.24	45.35	31.78

Legend: HMF – hydroxymethylfurfural, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Table 5 Physico-chemical quality of multifloral honeys

	Water content [g $100g^{-1}$]	Water activity	HMF [mg kg^{-1}]	pH	Free acidity [meq kg^{-1}]	Diastase [DN]
n	16	16	15	16	16	16
average	17.41	0.558	4.01	4.66	24.27	25.81
SD	2.25	0.036	1.71	0.45	13.24	10.38
min	14.40	0.504	0.77	3.80	10.40	14.85
max	21.90	0.629	7.05	5.20	50.20	52.36
coef. var. [%]	12.92	6.50	42.64	9.62	54.56	40.22

Legend: HMF – hydroxymethylfurfural, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Microbiological quality of honeys

The results from microbiological analyses of acacia, other unifloral and multifloral honeys are recorded in the Table 6, 7 and 8.

Coliform bacteria were not detected in all blossom honey samples. Generally, the presence of coliform bacteria is not allowed in food products and indicates faecal contamination. However, sometimes coliform bacteria can be found in fresh honey samples, as in study of **Omafuvbe and Akanbi (2009)**, who found these bacteria only in one sample at low count (1.48 log CFU g^{-1}). The predominant microorganisms found in honey are derived from the nectar and the honey bee (**Chaven, 2014**).

TPC was detected in all samples instead of one acacia honey. TPC ranged from 1.00 to 3.48 log CFU g^{-1}. The higher TPC value (above 2.00 log CFU g^{-1}) was exceeded in two acacia honeys, one sample of clover and rape honey and five samples of multifloral honeys. **Różańska (2011)** found similar TPC values in unifloral and multifloral Polish honeys; they ranged from 1.00 to 4.88 log CFU g^{-1}. **Omafuvbe and Akanbi (2009)** found TPC between 3.00 and 3.70 log CFU g^{-1}. According to **Snowdon and Cliver (1996)** bacterial numbers of finished honeys tends to range from 1 to 5000 CFU g^{-1} (0-3.70 log CFU g^{-1}), with lower numbers possible with additional industrial treatment.

Total plate count of anaerobic bacteria was detected only in 2 samples of acacia honey (14.29%), in 1 chestnut honey (12.50% of other unifloral honeys) and in 8 samples of multifloral honeys (50.00%). These values ranged from 1.00 log CFU g^{-1} (in chestnut honey) to 2.89 log CFU g^{-1} (in multifloral honey). Isolates were not identified at genus level. Some lactic acid bacteria (LAB) belong to the anaerobic bacteria and **Olofsson and Vásquez (2008)** discovered some LAB of the genera *Lactobacillus* and *Bifidobacterium* in the honey stomach of the honeybee and these organisms can end up eventually in the honey; they suggested that honey shall be considered as a fermented food product because of the LAB involved in honey production. According to **Snowdon and Cliver (1996)**, *Pseudomonas* sp. and *Micrococcus* sp. might also be found in the honey samples. These bacteria are aerobic generally and non-sporulating, but tolerant to external unfavourable factors. **Longaric *et al.* (2011)** identified isolates from the honey samples as *Comamonas* sp. and *Acinetobacter* sp. that are aerobic bacteria, too. The most osmotolerant bacteria (i. e. those which can survive in high sugar concentration) are staphylococci (**Cooper, 2005**).

Table 6 Microbiological quality of acacia honeys [log CFU g^{-1}]

	TPC	TPCan	SB	Yeasts	MFF
n	13	2	6	6	7
average	1.51	1.41	1.67	1.22	1.24
SD	0.47	0.21	0.76	0.39	0.40
min	1.00	1.26	1.26	1.00	1.00
max	2.42	1.56	3.19	1.95	2.07
coef. var. [%]	30.83	15.04	45.32	31.71	32.22

Legend: CFU – colony forming unit, TPC – total plate count, TPCan – total plate count of anaerobic microorganisms, SB – sporulating bacteria, MFF – microscopic filamentous fungi, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Table 7 Microbiological quality of other unifloral honeys [log CFU g^{-1}]

	TPC	TPCan	SB	Yeasts	MFF
n	8	1	1	4	4
average	1.75	1.00	1.00	1.48	1.58
SD	0.57	ND	ND	0.71	1.04
min	1.00	1.00	1.00	1.00	1.00
max	2.85	1.00	1.00	2.50	3.13
coef. var. [%]	32.58	ND	ND	48.06	65.83

Legend: CFU – colony forming unit, TPC – total plate count, TPCan – total plate count of anaerobic microorganisms, SB – sporulating bacteria, MFF – microscopic filamentous fungi, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Table 8 Microbiological quality of multifloral honeys [log CFU g^{-1}]

	TPC	TPCan	SB	Yeasts	MFF
n	16	8	10	8	11
average	1.96	1.90	1.66	1.54	1.34
SD	0.70	0.84	0.72	0.61	0.51
min	1.08	1.00	1.00	1.00	1.00
max	3.48	2.89	2.82	2.73	2.79
coef. var. [%]	35.61	44.28	43.07	39.31	38.23

Legend: CFU – colony forming unit, TPC – total plate count, TPCan – total plate count of anaerobic microorganisms, SB – sporulating bacteria, MFF – microscopic filamentous fungi, n – number of samples with measured values, SD – standard deviation, min –minimum, max – maximum, coef. var. – coefficient of variation

Sporulating bacteria count was detected in 6 acacia honeys (42.86%), in 1 rape honey (12.50% of other unifloral honeys) and in 10 multifloral honeys (62.50%). Counts of sporulating bacteria ranged from 1.00 log CFU g^{-1} (in rape honey) to 3.19 log CFU g^{-1} (in acacia honey). **Omafuvbe and Akanbi (2009)** found sporulating bacteria ranged from 2.90 to 3.30 log CFU g^{-1}. **Tolba et al. (2007)** isolated the sporulating bacteria from honey samples with counts ranged from 2.00 to 3.23 log CFU g^{-1} and identified them as *Bacillus pumilus*, *B. licheniformis*, *B. subtilis*, *B. fusiformis* and *Paenibacillus motobuensis*, with *Bacillus pumilus* the most frequently identified species present. Bacterial spores, particularly those in the *Bacillus* genus, are regularly found in honey (**Snowdon and Cliver, 1996**).

Yeasts were detected in 6 acacia honeys (42.86%), in 4 other unifloral honeys (50.00%) and in 8 multifloral honeys (50.00%). Yeasts ranged from 1.00 to 2.73 log CFU g^{-1}. Higher yeasts count (above 2.00 log CFU g^{-1}) was found in 1 sample of lime honey and 2 samples of multifloral honey. Nectar itself has a microbial community associated with bees and yeasts are the most frequent inhabitants of floral nectar (**Escuredo *et al.*, 2012**). Osmophile and sugar tolerant yeasts are a problem in the honey industry, because they can grow even at the limited level of water available in ripe honey (**Snowdon and Cliver, 1996**) and under the right environment, may grow and ferment honey resulting in the formation of alcohol and carbon dioxide and a sour taste from breakdown of alcohol to acetic acid and water (**Chaven, 2014**).

Microscopic filamentous fungi were found in 7 acacia honeys (50.00%), in 4 other unifloral honeys (50.00%) and in 11 multifloral honeys (68.75%). MFF counts ranged from 1.00 log CFU g^{-1} to 3.13 log CFU g^{-1} (in buckwheat honey). Higher MFF counts (above 2.00 log CFU g^{-1}) was detected in 1 sample of acacia honey, 1 sample of buckwheat honey and 1 sample of multifloral honey. Presence of microscopic fungi was not detected in 3 acacia honeys (21.43%), 1 clover and 1 rape honey (25.00% of other unifloral honeys) and in 2 multifloral honeys (12.50%). **Różańska (2011)** found relatively low number of yeasts and MFF in Polish blossom honeys, too; their number exceeded 2.00 log CFU g^{-1} sporadically. The similar results were published by **Martins *et al.* (2003)**, who detected microscopic fungi in 88.8% of honey samples and identified three genera of microscopic filamentous fungi (*Aspergillus*, *Penicillium* and *Mucor*) and two genera of yeasts (*Saccharomyces* and *Candida*).

CONCLUSION

We tested three groups of blossom honey (acacia, other unifloral and multifloral), mainly originated in Slovakia. Three samples of multifloral honey exceed at least one of the physico-chemical parameters limit value. HMF and diastase values of all evaluated samples were in accordance with limit value. It indicates the correct way of honey processing and storage. Overall, the good quality of tested honey samples was found. Ripe honey of good quality has the unique physico-chemical properties that are unfriendly to vegetative bacterial cells. Probably, they are destroyed gradually during the maturation and storage and we can isolate some of the most tolerant in relatively fresh honeys. Multifloral honey comes from nectar of various plants. Consequently, honey contains wide range of chemical

substances, and various microorganisms come to the honey. That is probably the reason why all evaluated microbial groups were found in higher frequency in the multifloral honey compared to the acacia and other unifloral honey.

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