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REGULAR ARTICLE

MICROBIOLOGICAL PROPERTIES AND ANTIMICROBIAL EFFECT OF SLOVAKIAN AND POLISH HONEY HAVING REGARD TO THE WATER ACTIVITY AND WATER CONTENT

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ABSTRACT

The present of this study aimed to characterize forty honeys from apiarists available in the Slovakian and Polish apiarists in respect to microbial quality. The chemical parameters as water activity and water content of each honey sample were obtained to differentiate them, because these two factors are important of microorganisms contamination. Furthermore, the antimicrobial effect against two pathogenic bacteria (*Escherichia coli* and *Bacillus cereus*) and one yeast (*Saccharomyces cerevisiae*) was also studied. Concerning the chemical

parameters, honey samples were found to meet European Legislation (EC Directive 2001/110) except for water content of four samples. Microbiologically, the commercial quality was considered good and all samples showed to be negative in respect to safety parameters. The antimicrobial activities of honey samples were tested by 10%, 25% and 50% (by mass per volume) concentration. The strongest antimicrobial effect was shown by 50% honey concentration against *Escherichia coli.*

Keywords: Honey, microbiological contamination, antimicrobial activity, pathogens, water activity, moisture

INTRODUCTION

Honey, as most natural products, may have a large variance in therapeutic components depending on its origin. Thus, the floral source of honey plays an important role on its biological properties. For example, Manuka honey from New Zealand is recognized for its therapeutic properties. The composition of honey has been shown to depend largely on its floral source. In consequence, it would not be surprising that the provenance of honey could determine its antibacterial properties. It is also possible that the mixing of honeys affect their antibacterial activity since honeys with lower antibacterial activities may mask the higher antibacterial activity of other honeys **(Basualdo et al., 2007).**

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics **(Finola et al., 2007).** On the other hand, EU legislation lacks specifications concerning microbial contamination and hygiene of the product. In fact, numerous studies have been reported on the physicochemical parameters of honeys from all over the world **(Azeredo et al., 2003; Finola et al., 2007; Kucuk et al., 2007; Al et al., 2009)**, but microbial contamination studies are rare and are essentially devoted to *Clostridium botulinum* **(Iurlina and Fritz, 2005; Nevas et al., 2002, 2005; Finola et al., 2007).**

Honey has several sources of microbial contamination. Primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar, and are somewhat difficult to eliminate. On the other hand, secondary sources, due to honey handlers and processing, are easier to control by the application of good manufacturing practices **(Snowdon and Cliver, 1995).** The major microbial contaminants include moulds and yeasts, as well the spores of *Bacillus* spp. and *Clostridium* spp. **(Snowdon and Cliver, 1995)**, being their counts indicative of honeys' commercial quality and safety.

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey **(Mandal and Mandal, 2011).**

The present of this study aimed to characterize forty honeys available in the Slovakian and Polish apiaries in respect to microbial quality. The chemical parameters as water activity and water content of each honey sample were obtained to differentiate them, because these two factors are important of microorganisms contamination. Total count of aerobic bacteria, anaerobic bacteria, moulds and yeasts were the microbial contaminants of interest. Furthermore, the antimicrobial effect against pathogenic bacteria and yeast was also studied.

MATERIAL AND METHODS

Honey samples

Forty honeys where purchased from local market and apiarist from Slovakia and Poland and left at room temperature until further analysis. All tested honey samples are in table 1.

Table 1 Characterization of honey samples

Sample	Slovakia	Poland
1.	Polyfloral, wood, 2007	Polyfloral, 2010
2.	Polyfloral, wood, 2010	Polyfloral, 2009
3.	Polyfloral, wood, 2010	Polyfloral, 2010
4.	Polyfloral, wood, 2009	Polyfloral, 2010
5.	Polyfloral, wood, 2008	Polyfloral, 2010
6.	Sunflower, 2011	Buckwheat, 2010
7.	Sunflower, 2011	Buckwheat, 2010
8.	Sunflower, 2011	Buckwheat, 2010
9.	Sunflower, 2011	Buckwheat, 2009
10.	Sunflower, 2011	Buckwheat, 2008
11.	Honeydew, 2009	Heather, 2010
12.	Honeydew, 2008	Heather, 2009

13.	Honeydew, 2010	Heather, 2010
14.	Monofloral, *Tilia platyphyllos,* 2009	Monofloral *Solidago* L., 2010
15.	Monofloral, *Tilia platyphyllos,* 2009	Monofloral *Phacelia tanacetifolia* Benth., 2010
16.	Monofloral, *Tilia platyphyllos,* 2009	Monofloral *Phacelia tanacetifolia* Benth., 2010
17.	Monofloral, *Robinia pseudoacacia L.,* 2009	Monofloral *Phaseolus coccinneus*, 2010
18.	Monofloral, *Robinia pseudoacacia L.,* 2009	Monofloral *Taraxacum officinale*, 2010
19.	Blossom nectar, 2010	Blossom nectar, 2010
20.	Bloosom nectar, 2010	Bloosom nectar, 2010

Microbial contamination

Ten grams of each honey sample were homogenized into 90 mL of solution physiological. Decimal dilutions were made into the same solvent. Aerobic bacteria were counted onto meat peptone agar and incubated at 30 °C for 48-72 h. Anaerobic bacteria were cultivated on Anaerobic agar at 30 °C for 48-72 h. Microscopic fungi and yeast were cultivated on Czapek - Dox agar at 25 °C for 5-7 days. Microbial counts were expressed as colony-forming units per gram of honey ($cfu.g^{-1}$).

Antimicrobial activity

Honey solutions were prepared in three concentrations: 50, 25 and 12.5 % (by mass per volume). The samples of each honey (1 g) and sterile water were stored at 37 °C for 30 min before mixing, to facilitate homogenization. The 50 % (by mass per volume) solutions thus prepared were diluted to 25 and 12.5 %. The samples were assayed immediately after dilution. Honeys were screened for their antimicrobial activity, according to the agar well diffusion method and disc diffusion method proposed by the CLSI (former NCCLS) against the following three reference strains: (1) *Escherichia col*i, (2) *Bacillus cereus* and (3) *Saccharomyces cerevisiae*. All the above microorganisms were grown (100 mL) in Muller Hinton broth at 37°C for 18 h (Biomark^{TM}, Pune, India). Muller Hinton agar (Biomark^{TM}, Pune, India) for bacteria and Sabouard agar (Biomark^{TM}, Pune, India) for yeast (150 mL) plates were poured and stored at 4°C for 24 h followed by agar well opening on plates and disc diffusion method (6 mm diameter). Antibacterial activity was assessed by measuring the

diameter of the inhibition zones surrounding the wells and discs. Two replicate plates were used per each concentration of honey and the experiment was repeated twice.

The water content and water activity of each honey samples were measured simultaneously.

Water activity

Water activity of each sample was measured with LabMaster-aw (Novasina, Pfaffikon, Switzerland). Every sample was analyzed in three parallel determinations.

Water content

The water content was detected using a portable refractometer (ATAGO®, Tokyo, Japan).

Statistical analysis

The statistical processing of the data obtained from all studies was implemented by means with STATGRAPHICS 5 software. Experimental results were expressed as means, standard deviation (SD) and coefficient of variability (CV).

RESULTS AND DISCUSSION

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. 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. The microbes of concern in honey are primarily yeast and spore-forming bacteria. Total plate counts from honey

samples can very 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 (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., 2007).**

Table 2 Microbial (log cfu.g^{-1}) and chemical properties of honey samples

Honey samples	**Slovakia**					**Poland**				
	AMB	**AB**	**MFY**	**a_w**	**Wc %**	**AMB**	**AB**	**MFY**	**a_w**	**Wc %**
1.	ND	ND	1.50	0.522	17.9	1.00	ND	1.30	0.509	17.0
2.	ND	ND	ND	0.599	18.1	1.10	ND	1.70	0.598	19.4
3.	ND	ND	ND	0.597	19.5	1.54	ND	1.90	0.608	20.4
4.	ND	ND	1.23	0.500	17.8	1.88	1.00	2.13	0.590	19.6
5.	ND	ND	ND	0.526	18.2	1.17	ND	2.24	0.568	16.6
6.	ND	ND	ND	0.542	16.5	1.30	ND	ND	0.559	19.2
7.	ND	ND	1.25	0.523	16.7	1.00	ND	ND	0.572	17.4
8.	ND	ND	1.15	0.659	15.9	1.40	ND	1.18	0.585	17.5
9.	ND	ND	1.12	0.526	17.9	1.82	ND	ND	0.597	18.4
10.	ND	ND	ND	0.528	18.0	1.00	ND	1.00	0.561	17.2
11.	ND	ND	ND	0.522	19.2	1.00	ND	1.00	0.579	18.1
12.	ND	ND	ND	0.599	17.8	1.30	ND	1.54	0.592	20.3
13.	ND	ND	ND	0.597	16.8	1.00	ND	1.17	0.598	20.3
14.	ND	ND	ND	0.500	18.0	1.65	ND	1.54	0.571	18.6
15.	ND	ND	1.25	0.526	17.8	1.40	1.54	1.40	0.574	18.4
16.	ND	ND	1.31	0.542	16.8	1.30	ND	1.00	0.556	18.1
17.	ND	ND	ND	0.523	17.8	1.54	ND	1.40	0.575	16.9
18.	ND	ND	ND	0.659	19.2	1.40	ND	1.48	0.594	21.6
19.	ND	ND	ND	0.526	17.5	1.54	ND	1.17	0.530	18.8
20.	ND	ND	ND	0.528	16.9	1.00	ND	1.30	0.574	18.6

AMB-aerobic mesophiles bacteria, anaerobic bacteria, MFY- microscopic filamentous fungi and yeasts, a_w- water activity, Wc- water content [%], ND-not determined

Levels of microbial contamination of honey samples are presented in Table 2. Levels of quantification for the commercial quality parameters (aerobic and anaerobic mesophilic bacteria, microscopic filamentous fungi and yeasts) in the analyzed honey samples were not detected are generally lower than those reported by other authors **(Iurlina and Fritz, 2005; Finola et al., 2007).** Also in table 2 we reported results of water activity and water content, which are factors for growth of microorganisms.

In comparison of Slovak and Polish honey we found the better microbiological quality of Slovakian honey in microbiological properties as number of aerobic mesophiles bacteria. Number of anaerobic bacteria in Slovakian honey we not found and in Polish honey we found only in the two samples. All followed samples of Slovakian bee honey comply with the requirements for honey on water activity and water content. Four Polish samples of honey do not correspond with Europe Union standard for honey in water content.

From the microbiological point of view, the low count of yeast and fungi is indicative of an appropriate management of apiaries. From this point of view, it can be said that the quality of the analyzed honeys is good, which facilitates its national and international commercialization.

The agar well diffusion method and disc diffusion method was used to ascertain the antimicrobial activity of the honey samples against two bacteria (*Escherichia coli* ATCC 14948; *Bacilus sutillis* ATCC 6633) and one yeast (*Saccharomyces cerevisiae*) (table 3).

Honeys from diverse floral sources were screened against *Escherichia coli* (ATCC 14948) and *Bacillus subtilis* (ATCC 6633) using the broth microdilution method. The best antimicrobial effect in this study were found in 50% concentration of honey against these bacterial strains **(Brudzinsky and Kim, 2011).**

The antibacterial properties of honey have been reviewed extensively during the last years in multiple studies all over the world. In our study each bacteria and yeast tested exhibited different sensitivities to each of the test honeys. Several authors have concluded that major antibacterial factors in honey are hydrogen peroxide, catalase and glucose oxidase level **(Weston et al., 2000).** Non-peroxide factors may also contribute to antimicrobial properties of honey such as lysozyme, phenolic acids and flavonoids **(Weston et al., 2000).** Flavonoids and other phenolic components in nectar have antioxidant capacity and inhibit growth a wide range of gram negative and gram positive bacteria. Moreover, several authors have concluded that honey from certain plants has better antibacterial activity than that of others. Also, it has shown that there can be a large variation in the activity of different samples from the same plant source **(Silici et al., 2010).**

Table 3 The statistical indicators of antimicrobial activity (in mm) of Slovak and Polish honey

		Slovak honey						Polish honey					
		n	x	S.D.	CV%	min	max	n	x	S.D.	CV%	min	max
well diffusion method													
E. coli													
	12.5	40	4.05	1.55	38.31	2	7	40	7.0	3.30	47.10	1	13
	25	40	7.4	1.81	24.44	5	11	40	9.38	3.22	34.39	1	16
	50	40	12.18	1.95	15.99	8	16	40	13.58	3.48	25.62	6	22
B. cereus													
	12.5	40	2.9	1.21	41.91	1	5	40	2.4	2.84	118.53	0	13
	25	40	5.6	1.68	29.94	2	9	40	4.05	3.46	85.34	0	13
	50	40	9.7	2.64	27.25	4	14	40	6.64	4.37	65.76	0	14
S. cerevisiae													
	12.5	40	1.47	1.06	71.99	0	4	40	3.23	5.00	155.04	0	15
	25	40	3.22	2.03	63.0	0	7	40	3.41	4.66	136.64	0	15
	50	40	5.25	2.18	41.54	1	9	40	5.44	5.77	106.16	0	15
disc diffusion method													
E. coli													
	12.5	40	4.2	1.49	35.44	2	7	40	7.65	10.93	142.91	3	14
	25	40	7.38	1.81	24.50	5	11	40	7.25	1.97	27.19	5	11
	50	40	12.23	1.83	14.99	9	16	40	10.55	1.60	15.1	7	14
B. cereus													
	12.5	40	2.9	1.22	41.91	1	5	40	3.13	2.13	68.05	0	9
	25	40	5.43	1.74	32.03	2	9	40	6.05	2.66	43.96	0	10
	50	40	9.98	2.50	25.02	4	15	40	8.65	3.80	43.93	0	14
S. cerevisiae													
	12.5	40	1.48	1.06	71.99	0	4	40	1.05	1.43	136.13	0	5
	25	40	3.23	2.03	63.00	0	7	40	1.89	2.43	128.87	0	10
	50	40	5.25	2.18	41.54	1	9	40	2.53	3.33	131.81	0	15

n-number of samples, x-average, S.D.-standard deviation, CV-coefficient of variation, min-minimum, max-maximum

CONCLUSION

The study allowed the qualitative analysis of the honey samples collected from beekeepers in Slovakia and Poland. The experimental values of the chemical and microbiological parameters of honey demonstrate the following:

- The presence of microorganisms (especially, moulds) which cannot exceed the limit values. These facts, as well as the favourable conditions can lead to generating and developing mycotoxins.

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

- The chemical parameters were within the limits imposed by the present legislation, except 4 samples.

The best antimicrobial properties of honey were found in Slovak and Polish honey in 50% concentration against *Escherichia coli*.

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