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REGULAR ARTICLE

PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF HONEY FROM LIPTOV REGION

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ABSTRACT

The present study aimed to characterize twenty four honeys available in the Slovak apiarist in respect to their floral origins (rape, lime, honeydew honey), physicochemical and microbial parameters. Moisture content, ash, acidity and electrical conductivity were the parameters analyzed in each honey sample. Total bacteria count and coliform bacteria were the microbial contaminants of interest studied. Concerning the physicochemical parameters, all honey samples were found to meet European Legislation (EC Directive 2001/110) for all parameters. Microbiologically, the commercial quality was considered good and all samples showed to be negative in respect to safety parameters.

Keywords: honey, apiarist, physicochemical and microbiological properties

INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretion of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (**Codex standard for honey, 2001**).

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Internationally, honey quality criteria are specified in Regulatory Standards, compiled in a Codex Alimentarius standard which at present is under revision (**Bogdanov, 2004**).

The Codex Alimentarius Standard for honey quality includes several chemical and physical parameters, comprising moisture content, mineral content, acidity, hydroxymethylfurfural (HMF) content, diastase activity, apparent sugar content, and water insoluble solids content. These analyses help the food analyst to determine the ‘‘chemical’’ quality of the honeys analyzed (**Cantarelli *et al.*, 2008**).

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.

The present study aimed to characterize three honeys type from two apiarist available in the Liptov area from Slovakia in respect to floral nectar origin, physicochemical parameters and microbial properties.

MATERIAL AND METHODS

Honey samples

In this study three honey types: rape 8 samples, lime 8 samples and honeydew 8 samples from two different beekeepers (A, B) of Liptov region were analyzed.

Physicochemical analyses of honey in Department of Evaluation and Processing Animal Products and microbiological properties in Department of Microbiology were analyzed.

For physicochemical and microbiological evaluation the honey samples of removing the stencil (100 ml), well mixed one extracted 3 samples were taken from 3 different containers were obtained.

Honey samples before analyses were transferred to a liquid consistency in water bass and temperature in water bass does not exceed 50 °C.

Physicochemical analysis

The water contents, free acidity, electrical conductivity and ash were measured. The determination of water content (**AOAC, 1990**) was ascertained by refractometry, using a Refractometer HHR-2N (ATAGO®, Japan). All measurements were performed at 20 °C, after waiting for 6 min for equilibrium, and obtaining the corresponding % moisture (g.100 g^{-1} honey) from the refractive index of the honey sample by consulting a standard table for the purpose.

Free acidity was determined by potentiometric titration (**AOAC, 1990**). Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. Ten grams of honey were then dissolved in 75 mL of distilled water, and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

Electrical conductivity was determined by conductimetric assay (Mini-digi conductivimeter), from a solution containing 10 g of honey in 75 mL of distilled water **(Sancho *et al.*, 1992)**.

Ash content was measured by **STN 570190**.

Microbiological analysis

We analyzed all of the honey samples using basic and additional microbiological analyses. The basic microbiological analyses consisted of total plate counts and coliform bacteria counts. The numbers of these microbial groups in the honeys tested and the values of CA SR (2011) were compared. In these analyses we followed the established STN: **STN EN ISO 4833 (1997)** for TPC and **STN ISO 4832 (1997)** for coliform bacteria.

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 (x), standard deviation (SD), standard error (SE) and coefficient of variability (CV). Confidence limits were added at $P < 0.05$; $P < 0.01$; $P < 0.001$.

RESULTS AND DISCUSSION

Tables 1, 2, 3 shows the results obtained for the physicochemical parameters analyzed in the samples of honey. All samples were found to meet honeys quality European Legislation **(EC Directive 2001/110)** in all parameters.

Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year (**Acquarone *et al.*, 2007**). High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. Moisture contents of honey samples ranged from 15.9 to 17.2, which are well below to the imposed limit of 20 % (**Codex Standard for honey, 2001**). Mean values of moisture content of honey samples in the study of **Mahmoudi *et al.*, 2012** were in the limit of acceptable international standards of honey moisture content (≤ 20 %). In the study of **Kahraman *et al.*, 2010**, 4.29 % of samples were in an unacceptable range. Similar results as our study (**Nanda *et al.*, 2003; Duman Aydin *et al.*, 2008**) and higher (**Przybylowski and Wilczynska, 2001; Rodriguez *et al.*, 2004; Guler, 2005**) results were detected in previous studies. The different moisture content of honey depends on harvest season, the degree of maturity reached in the hive and moisture content of original plant (**Finola *et al.*, 2007**).

These results are indicative of good storage ability of these honeys, since high moisture content could lead to fermentation during storage. Electrical conductivity and free acidity values are also within the limits (lower than 0.8 $mS.cm^{-1}$ and 50 $meq.kg^{-1}$, respectively).

Kačániová *et al.*, 2012 found that all followed samples of Slovakian bee honey comply with the requirements for honey on water activity and water content.

The free acidity of honey may be explained by taking into account the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions, such as phosphate. High acidity can be indicative of fermentation of sugars

into organic acids. None of the samples exceeded the limit allowed, which may be taken as indicative of freshness of all honey samples. The acid content in honey is characterized by the free acidity. The measure and is useful for the evaluation of honey fermentation. A maximum of 40 meq.kg^{-1} is defined by the current standards. Furthermore it is helpful for the authentication of unifloral honeys and especially for the differentiation between nectar and honeydew honeys (**Sanz *et al.*, 2005**).

None of the analyzed honey samples showed electrical conductivity values superior to 0.8 mS.cm^{-1} (variation between 0.17 and 0.35 mS.cm^{-1} in rape honey, 0.21 and 0.28 mS.cm^{-1} in lime honey and 0.84 and 0.93 mS.cm^{-1} in honeydew honey), suggesting that all samples are from nectar honey, which is corroborated by the content of total ashes inferior to 0.6% (**Codex Standard for honey, 2001**)

Electrical conductivity reflect the mineral and acid contents of honey. The electrical conductivity is used to distinguish between floral and honeydew honeys according to the present standards. It is also the most important physicochemical measured for the authentication of unifloral honeys (**Mateo and Bosch-Reig, 1998**).

The statistical significant differences of physicochemical properties between beekeepers A and B are provided in table 1, 2, 3.

Table 1 Physicochemical characteristics of rape honey

	Refraction		**Moisture content %**		**Free acidity meq.kg^{-1}**		**Electrical conductivity mS.cm^{-1}**		**Ash g.100 g^{-1}**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
x	1.495	1.491	16.53	18.07	13.33	24.67	0.17	0.39	0.04	0.101
SD	0.01	0.01	0.12	0.12	0.76	0.58	0.02	0.02	0.01	0.02
SE	0.01	0.01	0.06	0.07	0.44	0.33	0.01	0.009	0.01	0.01
CV%	0.02	0.03	0.69	0.64	5.73	2.34	10.92	3.85	2.5	3.5
T- test	$P < 0.05$		$P < 0.01$		$P < 0.001$		$P < 0.001$		$P < 0.001$	

Table 2 Physicochemical characteristics of lime honey

	Refraction		**Moisture content %**		**Free acidity meq.kg^{-1}**		**Electrical conductivity mS.cm^{-1}**		**Ash g.100 g^{-1}**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
x	1.488	1.496	19.40	16.40	16.20	35.37	0.21	0.28	0.05	0.07
SD	0.01	0.01	0.2	0.2	0.26	0.55	0.03	0.02	0.063	0.062
SE	0.01	0.01	0.12	0.12	0.15	0.32	0.02	0.009	0.036	0.036
CV%	0.034	0.03	1.03	1.02	1.63	1.56	12.59	5.52	5.57	3.32
T- test	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.05$		$P < 0.001$	

Table 3 Physicochemical characteristics of honeydew honey

	Refraction		Moisture content %		Free acidity meq.kg^{-1}		Electrical conductivity mS.cm^{-1}		Ash g.100 g^{-1}	
	A	B	A	B	A	B	A	B	A	B
x	1.495	1.493	16.47	17.20	24.00	27.20	0.84	0.93	0.06	0.08
SD	0.01	0.01	0.23	0.2	1.32	0..26	0.01	0.01	0.04	0.02
SE	0.01	0.01	0.13	0.12	0.76	0.15	0.01	0.01	0.02	0.01
CV%	0.04	0.04	1.4	1.16	5.51	0.97	4.17	1.95	2.79	1.30
T- test	P > 0.05		P > 0.01		P < 0.05		P < 0.01		P < 0.05	

The microbiological characteristics of honey samples obtained from Liptov region are presented in Table 4. The results showed level of microbial counts for the honey samples.

The standard plate counts (SPC) were found in low numbers in most samples of honey with a minimum count of 10 cfu.g^{-1} and a maximum 3.10^{2} cfu.g^{-1}. Total coliforms were not detected in any of the honey sample, similar as in study **Kačániová *et al.* (2007)** and **Adenekan *et al.* (2010)**. This may be explained by the evidence that honey is well preserved against bacteria so that these microorganisms would not survive unfavorable conditions. Few samples of honey contained detectable levels of yeasts, below 100 cfu.g^{-1}. This range may approach data reported by **Snowdon and Cliver (1996)**. The microorganisms that may be found in honey are mostly yeasts and spore-forming bacteria, but no disease causing bacteria species had been detected in honey samples. Therefore, honey has inherent antimicrobial properties that can delay or inhibit growth of many microorganisms.

Table 4 Total count of bacteria in cfu.g^{-1}

	Rape honey		T-test	Lime honey		T-test	Honeydew honey		T-test
	A	B		A	B		A	B	
X	46.67	233.33	P > 0.05	53.33	90.00	P > 0.05	10.00	80.00	P > 0.05
SD	40.42	152.75		40.41	112.69		0.00	10.00	
SE	23.33	88.19		23.33	65.06		0.00	5.70	
CV%	86.6	65.47		75.77	125.22		0.00	12.50	

In our study total count of bacteria ranged from 46.67 to 233.33 cfu.g^{-1} in rape honey, from 53.33 to 90.00 cfu.g^{-1} in lime honey and from10.00 to 80 cfu.g^{-1} in honeydew honey. The highest microbiological quality in honeydew honey was analyzed. The statistical significant differences were not found.

In different study of microbiological properties of honey the mean total bacteria count for Ekiti honey samples was 1.5.10^{3} cfu.ml^{-1}, while the mean value of 5.5.10^{5} cfu.ml^{-1} was

obtained from the Lagos honey samples. The mean value of $1.6.10^6$ cfu.ml^{-1} was obtained for Ogun state honey samples and $2.0.10^5$ cfu.ml^{-1} for Ondo state honey samples. Osun state recorded the mean value of $3.5.10^5$ cfu.ml^{-1} while Oyo state had a mean value of $1.0.10^3$ cfu.ml^{-1}. The mean total coliform counts in honey samples from six states in southwestern Nigeria showed that the mean total coliform counts for Ekiti, Lagos and Ogun states were $2.4.10^5$ cells.100 ml^{-1}, 95.0 cells.100 ml^{-1} and 95.0 cells.100 ml^{-1} respectively. The honey samples from Ondo, Osun and Oyo states recorded 39.0 cells.100 ml^{-1}, $1.1.10^3$ cells.100 ml^{-1} and 95 cells.100 ml^{-1} respectively (**Adebayo and Davies, 2012**).

CONCLUSION

The study allowed the qualitative analysis of the honey samples collected from beekeepers in Slovakia of Liptov region. The experimental values of the physicochemical and microbiological parameters of honey demonstrate the following: The physicochemical parameters were within the limits imposed by the present legislation. The presence of microorganisms was within the limits imposed by the present legislation.

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