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HONEY CHARACTERISTICS AFTER EXTRACTION AND HALF-YEAR STORAGE

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ABSTRACT

The aim of the study was to analyze the fresh honey after extracting and after half-year storage at room temperature. Overall, we analyzed 10 samples of rape (*Brassica napus*) honey coming from district Vranov nad Toplou located in the eastern Slovakia. The analysis consisted of the evaluation of the physico-chemical parameters (water content, free acidity and electrical conductivity) and microbiological evaluation (total plate count (TPC), counts of coliform bacteria, lactic acid bacteria, sporulating microorganisms and microscopic fungi). Water content, free acidity and electrical conductivity were measured according to IHC (2009), namely these parameters were detected by refractometer, titration and conductometer, respectively. We used dilution plating method for microbiological analysis. Fresh rape honey contained 18.3 ±1.0% of water. Free acidity of fresh rape honey was 12.7 ±2.0 meq.kg^{-1} and electrical conductivity was 0.14 mS.cm^{-1}. After half a year of storage, water content and electrical conductivity decreased nonsignificantly and free acidity increased nonsignificantly. Stored honey samples meet the requirements of Decree 41/2012 and 106/2012. From microbiological point of view, fresh rape honey showed relatively high microbial counts. Mean values of TPC, sporulating microorganisms, lactic acid bacteria and yeasts exceeded 2.00 log cfu/g. All spotted microbial groups decreased in the stored honey comparing with the fresh honey. We found significant (p <0.01) differences of TPC, lactic acid bacteria and yeasts comparing the fresh and stored honey samples. Evaluating microbiological parameters, one sample of stored honey did not meet the requirements of Codex Alimentarius SR (2014). TPC exceeded the limit value. Based on the results we can conclude that all samples meet the requirements for good quality honey. Microbial counts in the honey decreased gradually. Probably, various microorganisms have important role in creation of the honey from the nectar of plants, but non-sporulating microorganisms die in the ripe honey.

Keywords: honeybee, rape, microorganism, electrical conductivity

INTRODUCTION

Today, honey is one of the last untreated natural foods and the consumption of honey differs strongly from country to country **(Bogdanov, 2015)**. Research and education in this field may be the good tools to rediscovery of these interesting products in our homes.

Honey is classified by its floral source from which the nectar is collected by the honey bee. Unifloral honey is made primarily from the nectar of one type of common flower in which the bees have acces **(Chaven, 2014)**.

Rape (*Brassica napus* L. and other species, hybrids and varieties) is largely cultivated in Europe for the seed, used for oil production. It is very attractive to bees both for nectar and pollen and in Central and Eastern European countries represents one of the most important spring sources, giving rise to large amounts of very pure unifloral honey **(Oddo et al., 2004)**.

Rape honey is popular in Slovakia because of its interesting properties. Rape honey has pleasant taste and it is suitable for formation of creamed honey.

In general, ripe honey is commonly considered to be relatively sterile foodstuff. The properties of honey that make it effective against bacterial growth are: high sugar content, low water activity, gluconic acid, which creates an acidic environment and hydrogen peroxide **(Khan et al., 2007)**. **Olofsson and Vásquez (2008)** suggested that honey be considered as a fermented food product because of the lactic acid bacteria (LAB) involved in honey production.

Nectar is converted into honey through a maturation process, in which a considerable water loss (40 to 70% of nectar initial weight) is the most prominent feature **(Ruiz-Argueso and Rodriguez-Navarro, 1975)**. The nectar sugars probably act as inducers for the resident honey stomach microbiota, enhancing their numbers, with the enhancement depending on the types of flowers that the honeybees visited; and the bacteria are added during the process by which nectar becomes honey **(Olofsson and Vásquez, 2008)**. Moreover, honey can play a bifidogenic role in detoxifying mycotoxins and decrease their detrimental effects including tissue necrosis **(Sidoo-Atwal and Atwal, 2012)**. **Vidová et al. (2013)** explain the bifidogenic substances as agents that can support the growth of *Bifidobacterium* sp. (*B. longum* ssp. *longum*,

B. breve, *B. pseudolongum*, *B. longum* ssp. *infantis* and *B. animalis* ssp. *lactis*) and *Lactobacillus* sp. (e. g. *L. acidophilus*, *L. casei*, *L. reuteri*, *L. plantarum*). **Ruiz-Argueso and Rodriguez-Navarro (1975)** isolated *Gluconobacter* sp., *Lactobacillus* sp., *Zymomonas* sp. and occasioanly several types of yeasts from ripening honey. **Olofsson and Vásquez (2008)** discovered LAB in the fresh honey, namely *Lactobacillus kunkeei*, *L. buchneri* and *Bifidobacterium asteroides* and they confirmed origin of these bacteria in honeybee stomach. **Endo and Salminen (2013)** found specific group of LAB - FLAB (fructophilic lactic acid bacteria) in fresh honey, namely *Lactobacillus kunkeei*. **Bogdanov (2015)** concludes that in general, honey shows prebiotic activity and fresh honey (about to 2-3 months old) contains probiotic bacteria.

The aim of the study was to compare the basic physico-chemical parameters and microbial counts in the fresh and stored rape honey from one Slovak region.

MATERIAL AND METHODOLOGY

We analysed 10 apiary samples of blossom honey. Samples were obtained directly from beekeepers from 7 villages of Vranov nad Toplou district. Figure 1 show the region in eastern part of Slovakia, where the samples originated, together with detailed sampling places (names of villages). Length of sampling area was 30 km. Samples originated mainly from nectar of rape fields with additional nectar of fruit trees. Detailed characteristic of evaluated samples is in Table 1. Samples were collected aseptically from honey vessels immediately after honey extraction. Samples were stored in refrigerator before the first analysis (2 weeks after extraction). Then, samples were stored at room temperature in dark place. The second analysis was undertaken after half a year of storage.

The evaluation was divided into 2 parts: physico-chemical and microbiological analysis.

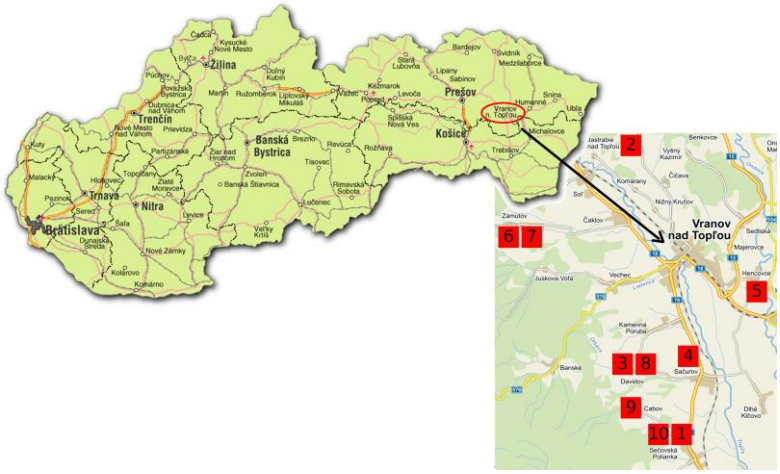

Figure 1 Sampling places on the map of Slovakia, 1 – 10 are number of samples.

Table 1 Characteristic of evaluated honey samples.

No.	Botanical or.	Geographical or.	Ext.
1	rape + fruit trees	Secovska Polianka	05/14
2	rape + fruit trees + pine	Jastrabie nad Toplou	05/14
3	rape + fruit trees	Davidov	05/14
4	rape + fruit trees	Sacurov	05/14
5	rape + fruit trees	Hencovce	05/14
6	rape + fruit trees	Zamutov	05/14
7	rape + fruit trees	Zamutov	05/14
8	rape + fruit trees	Davidov	05/14
9	rape + fruit trees	Cabov	05/14
10	rape + fruit trees	Secovska Polianka	05/14

Note: No. – number of sample, or. – origin, Ext. – date of extraction.

Table 2 Cultivation conditions used for microbiological analysis.

Microbial group	Medium	Inoculation	Conditions of cultivation		
			O_2 requirement	Temperature	Time
TPC	GTY	pouring	aerobic	30 °C	48-72 h
CB	VRBL	pouring	aerobic	30 °C	24 h
LAB	MRS	pouring[1]	aerobic[1]	37 °C	48-72 h
SM	NA 2	pouring[2]	aerobic	25 °C	48-72 h
MF	GYCH	pouring	aerobic	25 °C	5-7 days

Note: TPC – total plate count, cultivated on GTY – agar with glucose, tryptone and yeast extract (HiMedia); CB – coliform bacteria, cultivated on VRBL – violet red bile lactose agar (HiMedia); LAB – lactic acid bacteria, inoculated by [1]double-pouring (decrease of oxygen in medium), cultivated on MRS – de Man, Rogosa and Sharpe agar (HiMedia), SM – sporulating microorganisms, inoculated by [2]pouring – after heat shock (at 80 °C for 10 min), cultivated on NA – nutrient agar no. 2 (HiMedia); MF – microscopic fungi, cultivated on GYCH – agar with glucose, yeast extract and chloramphenicol (HiMedia).

Physico-chemical analysis consisted of water content, free acidity and electrical conductivity determinations. The parameters were detected according to **IHC (2009)**. Water content was measured by Abbe refractometer (Krüss). Free acidity was measured by titration as follows: We mixed honey (10 g) with distilled water (75 mL). We added 2 – 3 drops of phenolphthalein. The solution was titrated with 0.1 mol.L^{-1} sodium hydroxide to creation of pink colour. The consumption of 0.1 mol.L^{-1} sodium hydroxide was multiplied by 10 to expression of free acidity in meq.kg^{-1}. Electrical conductivity was measured by conductometer Mini-Digi Conductivity Meter OK-113 (Rudelkis) as follows: We dissolved an amount of honey, equivalent to 20 g anhydrous honey, in distilled water. We transferred the solution to a 100 mL flask and make up to final volume with distilled water. We measured conductanse (mS) of solution and multiplied by cell constant of the conductivity cell (cm^{-1}) to expression electrical conductivity in mS/cm. All physico-chemical measurements were performed in duplicate with mean value expression.

Microbiological analysis consisted of total plate count (TPC), counts of coliform bacteria, lactic acid bacteria (LAB), sporulating microorganisms and microscopic fungi determinations. We used dilution plating method. Basic dilution (10^{-1}) was made by homogenizing 5 g of the sample with 45 mL of saline solution with peptone (0.85% sodium chloride, 0.1% bacteriological peptone, pH 7.0 ± 0.2) for 30 min. Then, we prepared a 10^{-2} dilution according to the principle of the ten-fold dilution system. We inoculated the 10^{-1} and 10^{-2} dilutions for all spotted microbial groups onto sterile Petri dishes. Plates were cultivated under specific conditions. The characteristics of methods used are shown in Table 2. After cultivation, plate counts were calculated as cfu/g and coverted into the common logarithms and results were presented as log cfu/g. Counts of microscopic fungi were expressed separately for yeasts and separately for microscopic filamentous fungi. The detection limit of this method was 10 CFU.g^{-1} (1.00 log CFU.g^{-1}).

RESULTS AND DISCUSSION

The physico-chemical quality of evaluated honey after extraction and half-year storage is reported in Table 3.

Appearance of stored rape honey samples is showed in

Table 3 Physico-chemical quality of fresh and stored honey.

	Fresh honey (n = 10)	**Honey stored for 6 months** (n = 10)
Water content [%]	18.3 ±1.0	17.5 ±0.9
Free acidity [meq.kg^{-1}]	12.7 ±2.0	13.0 ±2.6
Electrical conductivity [mS.cm^{-1}]	0.14 ±0.02	0.13 ±0.02

Note: There were no significant differences between values in rows acording to the t-test (p <0.05).

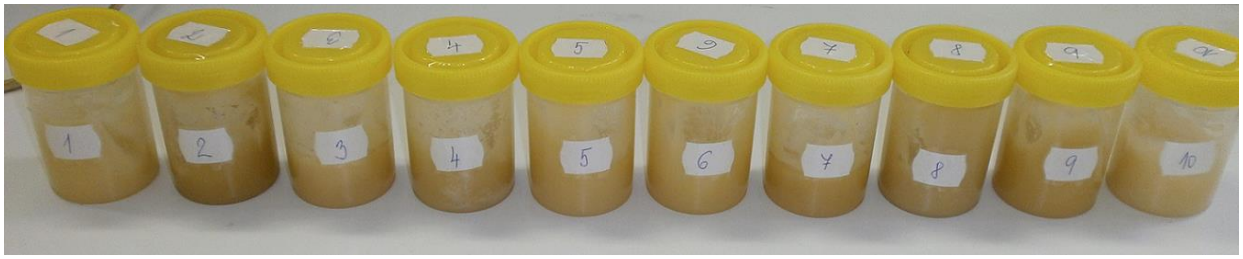

Figure 2 Samples of rape honey (photo: Bacikova, 2014).

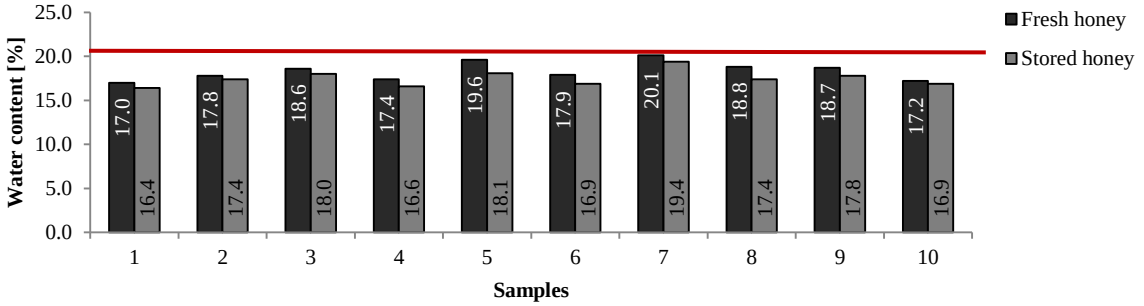

Figure 3 Water content in fresh and stored honey.

Table 4 Microbiological quality of fresh and stored honey [log cfu.g^{-1}].

	Fresh honey		Stored honey (after 6 months)	
	n	**mean ±SD**	**n**	**mean ±SD**
Total plate count	10	2.70^{a} ±0.78	10	1.62^{b} ±0.57
Coliform bacteria	2	1.24 ±0.34	0	ND
Sporulating microorganisms	8	2.02^{a} ±0.77	9	1.66^{a} ±0.57
Lactic acid bacteria [MRS]*	10	2.91^{a} ±0.73	8	1.24^{b} ±0.25
Yeasts	9	2.27^{a} ±0.39	3	1.26^{b} ±0.24
Microscopic filamentous fungi	9	1.92 ±0.61	1	ND

Note: n – number of samples with detectable microbial counts, SD – standard deviation, ND – not detected.
Different letters in the same row indicate statistical differences at p <0.01 according to the t-test.
* MRS is medium used for lactic acid bacteria isolation, but yeasts grown onto medium, too. From fresh honey samples we cultivated colonies mainly consisted of long rods and rod groups, occassionally of yeast cells (evaluated by microscopic view). From stored honey we cultivated only colonies consisted of yeast cells (evaluated by microscopic view).

the Figure 2. Colour of fresh rape honey is light yellow, sometimes lemon colour, it crystallizes very fast and result of crystallization is white colour, gentle crystals and solid, very hard consistency **(Kukurová et al., 2009)**. **Castro-Vázquez et al. (2012)** confirmed the importance of the use of low temperatures in order to keep the quality of physico-chemical parameters during the honey storage period. In general, recommended temperature for honey storage is max 20 °C.

During storage, water content, free acidity and electrical conductivity were without significant differences. Our results of physico-chemical quality are comparable with the data of 715 European rape honeys, reported by **Oddo et al., (2004)**, who found mean values of water content 17.0 ±1.1%, free acidity 10.3 ±2.1 meq.kg^{-1} and electrical conductivity 0.19 ±0.05 mS.cm^{-1}.

Water content is important parameter in term of honey shelf life. Figure 3 shows water content in evaluated samples of Slovak rape honey. Limit value is maximum 20% according to **Decree 41/2012** and **Codex Stan (2001)**. All samples (except sample no. 7 after extraction) met the requirements of the above mentioned standards.

Chaven (2014) states that honey with low water content (17.1 – 18.0%) and relatively high yeast spores content (max 10^3 cfu.g^{-1}, i. e. 3.00 log cfu.g^{-1}) will remain stable. Association of Slovakian beekeepers (SZV) constitutes **Standard for quality and grade of the SZV no. 1/2006**. All Slovakian beekeepers, who want to use the trademark SLOVENSKÝ MED (SLOVAKIAN HONEY) have to meet the additional criteria (e. g. water content: max 18%). Water content over 18% was found in 5 out of 10 (50%) fresh samples and in 2 out of 10 (20%) stored samples.

All honeys are acidic (pH 3.5 – 5.5), due to the presence of organic acids that contribute to the honey flavour and stability against microbial spoilage **(Bogdanov et al., 2004)**. Limit value for free acidity is 50 meq.kg^{-1} according to **Decree 41/2012** and **Codex Stan (2001)**. All samples met the requirement.

Electrical conductivity is parameter, which is suitable to diferrentiation between the blossom and honeydew honeys. Electrical conductivity of blossom honeys (except e. g. chestnut honey) must be maximum 0.8 mS.cm^{-1} **(Decree 106/2012; Codex Stan, 2001)**. Electrical conductivity of all evaluated samples was typical for blossom honeys. At present electrical conductivity is the most useful quality parameter for the classification of unifloral honeys, which can be determined by relatively inexpensive instrumentation **(Bogdanov et al., 2004)**.

The microbiological quality of fresh and stored rape honey is reported in Table 4. Significant (p <0.01) differences were found between fresh and stored honey in TPC, counts of LAB and yeasts.

Limit value for TPC is 10^2 CFU.g^{-1} (2.00 log CFU.g^{-1}), according to **Codex Alimentarius SR (2014)**. Obtained results of TPC in fresh and stored honeys are showed in Figure 4. Limit value for TPC was exceeded in 9 out of 10 (90%) fresh honey samples and in 1 out of 10 (10%) stored honey samples. **Sinacori et al. (2014)** found TPC values, which are comparable to the stored rape honey in this study. According to **Snowdon and Cliver (1996)**, counts of bacteria in finished honeys range normally from 1 to 5000 CFU.g^{-1} (0 – 3.70 log CFU.g^{-1}) and lower numbers are achieved by additional industrial treatment. We confirmed the importance of the honey age in microbial evaluating of the honey.

According to **Codex Alimentarius SR (2014)**, limit value for coliform bacteria in honey is 10^2 CFU.g^{-1} (2.00 log CFU.g^{-1}). Coliform bacteria were detected in 2 out of 10 (20%) fresh honey samples, only at low level, below limit value. After 6 months storage at room temperature, coliform bacteria were not detected in evaluated honeys. **Sinacori et al., (2014)** found the members of *Enterobacteriaceae* family only in 2 from 38 honeys. Coliform bacteria are present in honey

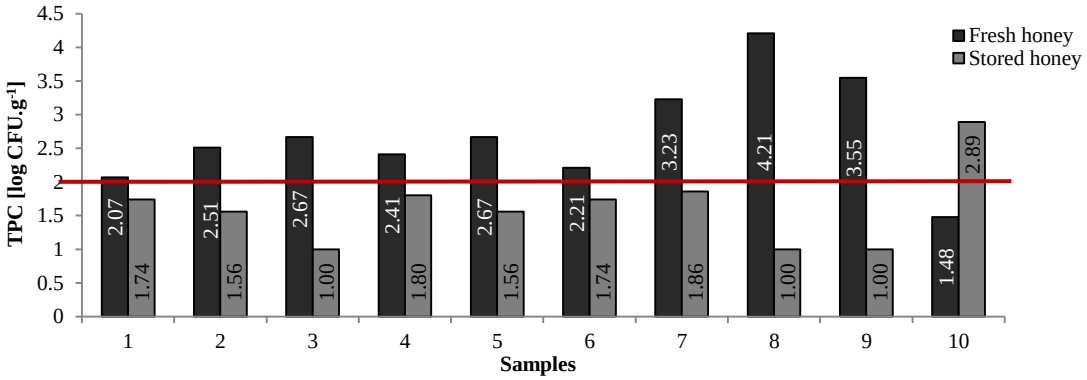

Figure 4 Total plate count in fresh and stored honey.

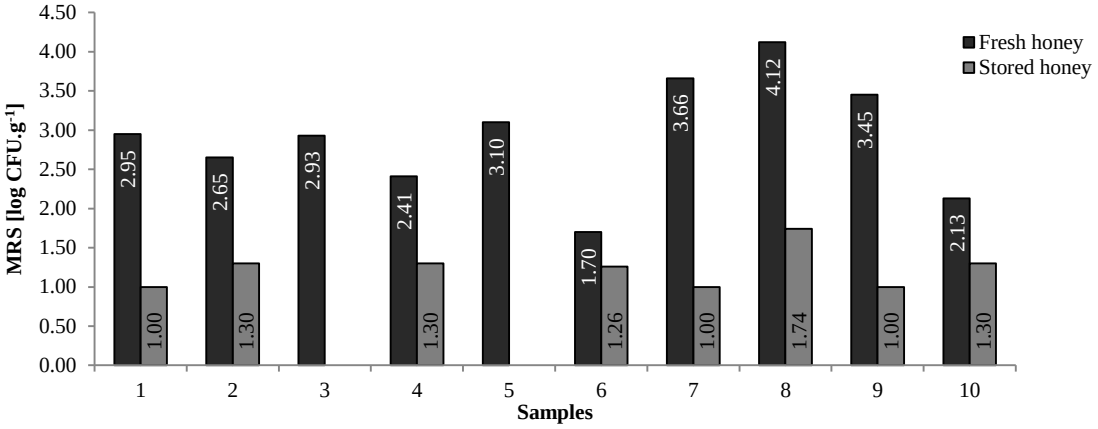

Figure 5 Microorganisms, cultivated on MRS medium, in fresh and stored honey.

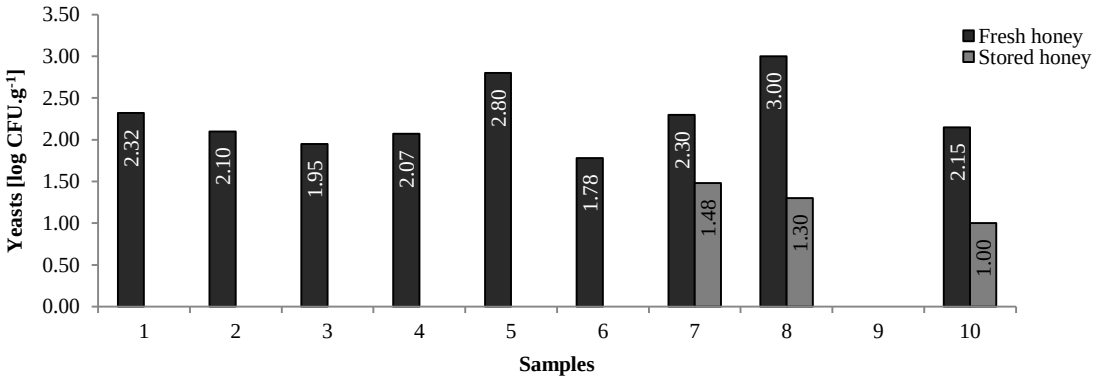

Figure 6 Yeasts in fresh and stored honey.

occasionally. In general, their high number in foodstuffs indicates faecal contamination.

Counts of sporulating microorganisms were without significant differences between the fresh and stored honey. **Sinacori et al. (2004)** analysed honey for sporulating microorganisms. They found no clostridia, but bacilli (*Bacillus amyloliquefaciens*, *B. subtilis*, *B. cereus*, *B. thuringiensis*, *B. licheniformis*, *B. megaterium*, *B. pumilus* and *B. simplex*) were frequent.

Obtained results of microorganisms, cultivated on MRS medium, in the fresh and stored honey are reported on Figure 5. Significant (p <0.01) difference was found between the fresh and stored honey. MRS medium is used for lactic acid bacteria cultivation. Cultivated isolates were checked by microscopic views. Cells from colonies originating in fresh honey were mainly the rods and group of rods. Occassionally the yeast cells were occurred. MRS medium is rich of nutrients and yeasts can grow on it, when they are not inhibited by other microorganisms (e. g. by LAB). From stored honey, we cultivated only yeasts on MRS medium. Nevertheless, we observed the presumptive LAB cultivated only from fresh honey samples. The results are in accordance with **Olofsson and Vásquez (2008)**, **Endo and Salminen (2013)** and **Bogdanov (2015)**. *Gluconobacter* and *Lactobacillus* populations decrease as ripening proceeds; the number of bacteria decreases from stomach nectar to higher-moisture honey to low-moisture honey **(Ruiz-Argueso and Rodriguez-Navarro, 1975)**.

Counts of yeasts in fresh and stored honey are showed on Figure 6. We observed significant (p <0.01) decrease of yeast count after storage. According to **Chaven (2014)**, wide variety of yeast may be recovered from unprocessed honey; it is predominantly osmophilic yeast varieties such as *Zygosaccharomyces rouxii* or *Z. bailii*, which are of relevant concern for honey processing. Presence of microscopic filamentous fungi was found in 9 out of 10 (90%) fresh honey samples and only in 1 out of 10 (10%) stored honey samples.

Fermentation is the only microbiological alteration to which honey is susceptible. Only osmophilic yeasts can grow in the high sugar concentrations, but their presence is ubiquitous in honey, nectar, hive interiors, dust and soils. Below 18% moisture content there is a little probability of fermentation, but even at concentration below 17.1% the risk of fermentation cannot be completely excluded. This aspect of fermentation depends on factors such as the quantity of yeasts and other growing factors – honey temperature and the distribution and availability of water following crystallization **(Krell, 1996)**.

The nutritional and health enhancing properties of unifloral honeys is quite a new field of research **(Bogdanov, 2015)**.

CONCLUSION

Obtained data of physico-chemical parameters (water content, free acidity and electrical conductivity) were typical for rape honey. In the stored honey, we recorded small decrease of water content and electrical conductivity and small increase of free acidity. However, the changes were without significant differences.

Presence of lactic acid bacteria in fresh rape honey from eastern Slovakia was confirmed by cultivating method with microscopic views.

Counts of spotted microbial groups decreased significantly during the storage. Probably, it indicates continued ripening in the honey package after extracting.

Next research plans are centered on testing of various fresh honeys and identification of microbial isolates, mainly presumptive members of lactic acid bacteria, by molecular-biological methods.

Presence of coliform bacteria, yeasts and primarily lactic acid bacteria in fresh honey can play an important role in the future trends of nutrition, but detailed research is needed because of potential risks.

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Map 1 : Slovak republic map [on line] [cit. 2015-03-15] Available at: http://th-engel-kanlitz.blog.cz

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