

## PHYSICOCHEMICAL CHARACTERIZATION OF NATURAL HONEYS FROM DIFFERENT REGIONS IN SLOVAKIA

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### Abstract

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This study is intended to determine the physicochemical characteristics of Slovakian honeys, and compare them with specifications described in the Codex Alimentarius Standard, the European Honey directive, the Slovak Codex Alimentarius and Slovak standard No. 1/2006. In addition, we tried to find out correlations between individual constituents of honeys. Physicochemical characterization was carried out following the harmonized methods dictated by the International Honey Commission IHC. Honey samples ( $n=50$ ) were collected from three honey types (blossom, blends, honeydew), and from the three main Slovakian geographical regions (named 'east', 'middle' and 'west'). The physicochemical evaluation included moisture, reduced sugars, proline, hydroxymethylfurfural (HMF), conductivity, diastase and invertase activity, pH and water activity, following the techniques proposed by the European Honey Commission (EHC). The moisture content in the honey varied from 15.26 to 17.65%, HMF levels ranged from 21.83 to 63.00 mg.kg<sup>-1</sup>, the diastase activity varied from 21.01 DN to 36.67, invertase activity varied from 121.73 to 164.11 U.kg<sup>-1</sup>, the proline content varied from 426.56 to 531.79 mg.kg<sup>-1</sup>, the fructose content values were found from 36.33 to 40.20 g.100g<sup>-1</sup>, the glucose content values ranged from 27.67 to 31.00 g.100g<sup>-1</sup>, the values of saccharose content were from 0.15 to 0.37 g.100g<sup>-1</sup>, the conductivity varied from 29.48 to 97.24 mS.cm<sup>-1</sup>, the pH value varied from 4.06 to 4.80 and the water activity content varied from 0.55 to 0.57. Significant differences ( $P \leq 0.05$ ) were found between HMF and fructose, glucose and saccharose and conductivity and pH, significant differences ( $P \leq 0.01$ ) between fructose and glucose and significant differences ( $P \leq 0.001$ ) were found between HMF and conductivity. Between blossom honey and honeydew honey were found statistical significant differences in HMF ( $P \leq 0.05$ ) and conductivity between honeydew honey and blossom honey ( $P \leq 0.001$ ). Among regions were determined statistical differences between HMF and invertase ( $P \leq 0.05$ ) and conductivity ( $P \leq 0.01$ ).

*Apis mellifera*, Slovakia, honey quality, Codex Alimentarius, European Honey Commission

The honey's chemical composition mainly depends on the vegetation sources from which it derives, climate, harvesting conditions and storage (Kačániová *et al.*, 2007a). It is well established that honey inhibits a broad spectrum of bacterial species and there are many reports of its bactericidal as well as its bacteriostatic activity (Kačániová *et al.*, 2008). Therefore, microbiological quality of honeys and hive environment are great Kňazovická *et al.*, 2008c). The water content (moisture) is an important quality

parameter for establishing honey shelf life, but not for the characterization of floral origin. However, depending on the season and the climate, even unifloral honeys might show differences in water content, affecting their physical (i. e. viscosity and crystallization) and sweetener properties (glucose/water ratio) (Persano-Oddo and Piro, 2004; Kačániová *et al.*, 2007b). Virtually absent in newly produced honey, hydroxymethylfurfural (HMF) is a byproduct of fructose decay, formed during storage or during heating.

Thus, its presence is considered the main indicator of honey deterioration (Krell, 1996). Fresh honey does not contain HMF, but this component can be present in honey as a result of chemical changes, providing an indication of the storage and processing (heating) of honeys. Therefore, HMF content is usually determined before any other parameters to assess the storage conditions/time, i. e. enzymatic activities and colour. Measuring of conductivity is an indirect way of measuring the mineral content of a honey (Kňazovická *et al.*, 2008a,b). As sugars in solution (the main component of honey) are poor conductors, any minerals present in the honey aid the ability of honey to conduct electricity. For honey, this is expressed as  $\text{mS.cm}^{-1}$  taken in a solution of honey and distilled water (at 20°C) where the dry matter of the honey makes up 20% of the weight of the solution. Typically flower honeys are less than 1.5  $\text{mS.cm}^{-1}$  while honeydew honeys are greater than 8.5  $\text{mS.cm}^{-1}$ . There are of course some exceptions to this and this feature is an aid in the identification of these honey types. Also blends of honeydew honeys and flower honeys will give an intermediate value (Bogdanov and Martin, 2002). The measurement of electrical conductivity (EC, 2002) is currently considered one of the most useful quality parameter for the classification of unifloral honeys, which can be determined by relatively inexpensive instrumentation. Based on an extensive survey of honeys from different parts of the world (EC 2002; Bogdanov 2002), EC was recently included in the new international standards for honey (EC, 2002; FAO, 2001), replacing the traditional ash determination. Honey is a typically acid product with pH ranging from 3.5 to 5.5, due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage. Gluconic acid is the main organic found in honeys, which is found with gluconolactone at different relationships. Free and total acidity, and pH, might discriminate unifloral honeys (Persano-Oddo and Piro, 2004). Proline is a free-aminoacid found

of honey, added by the bees during its production. Proline content, which is used as a ripeness criterion for honeys (Von der Ohe *et al.*, 1991) is characteristic from different unifloral honeys (Persano-Oddo and Piro, 2004), roughly correlated with the enzyme activity. The proline content among different unifloral honeys might be relatively high, and therefore is not possible to classify unifloral honey on the basis of proline content alone (Sanchez *et al.*, 2001; Persano-Oddo and Piro, 2004). Honey contains small amounts of different enzymes, the most important of which are diastase (alpha-amylase), invertase (alpha-glucosidase), glucose oxidase, catalase and acid phosphatase. In particular, diastase splits starch chains into dextrins and maltose; invertase is the enzyme responsible for converting saccharose to fructose and glucose which are the main sugars in honey. Enzymes as honey components have been the object of much research over the years: the primary interest was as a possible means of distinguishing between natural and artificial honeys, but diastase and invertase are also used as a measure of honey freshness, because their activity decreases in old or heated honeys (Dustmann *et al.*, 1985; Sancho *et al.*, 1992). The origin of invertase and diastase in honey is commonly attributed to the bee (Rinaudo *et al.*, 1973a,b). The nectar collected is mixed with secretions from the salivary and hypopharyngeal glands of foraging bees; then, in the hive, when the nectar is passed from bee to bee before being stored in the cells, more secretions are added, enabling nectar to ripen into honey. This process – and consequently the amount of added enzymes – depends on various factors such as age, diet and physiological stage of the bees, strength of the colony, temperature, abundance of nectar flow, etc. (Browers, 1983; Huang and Otis, 1989). Standards for honeys are set internationally by the European Honey Directive and the Codex Alimentarius (Table I) (FAO, 2001).

The aim of this work was to do a broad evaluation of the physicochemical quality of Slovakian ho-

I: Physicochemical characteristics of honey and limits given by the legislation of the Slovak Codex Alimentarius and Slovak republic (SR) standards No. 1/2006 and Codex Alimentarius (CA)

Characteristic	Limit SR	Limit CA
Moisture (%)	max. 18	max. 20
HMF ( $\text{mg.kg}^{-1}$ )	max. 20	max. 40
Proline ( $\text{mg.kg}^{-1}$ )	ND	180
Fructose, glucose ( $\text{g.100g}^{-1}$ )	min. B 60; BL, H 45	min. B 60; BL, H 45
Saccharose ( $\text{g.100g}^{-1}$ )	max. 5	max. 5
Conductivity ( $\text{mS.cm}^{-1}$ )	max. B,BL 0.8; min. H 0.8	max. B,BL 0.8; min. H 0.8
IA ( $\text{U.kg}^{-1}$ ), DA (DN)	min. 8	min. 8
pH	ND	ND
$a_w$	ND	ND

ND – not defined, B – blossom, BL – blends, H – honeydew, IA – Invertase activity, DA – Diastase activity

neys, in order to set the authenticity of the product, to see if the region has influence on natural honey composition.

## MATERIALS AND METHODS

### Honey sampling

50 honey samples were collected. These samples were mostly obtained from non-professional beekeepers, 16 from the blossom (sample 1–16), 17 from the blends (sample 17–33) and 17 from the honeydew types (sample 34–50). The East (17; sample 1–5, 17–22, 34–39), West (16; sample 6–10, 23–28, 40–44) and Middle (17; sample 11–16; 29–33, 45–50) Slovakian regions were equally represented in the groups. Samples were collected from separate hives within one month after extraction during July 2007 to August 2007. All the samples were stored between 0°C and 4°C. The physicochemical properties were determined according to methods in agreement with the EU (Krell, 1996).

### Physico-chemical analyses

Fifty honey samples from different Slovakian regions were analyzed at their moisture content (%), HMF ( $\text{mg} \cdot \text{kg}^{-1}$ ), proline ( $\text{mg} \cdot \text{kg}^{-1}$ ), reducing sugars as fructose ( $\text{g} \cdot 100\text{g}^{-1}$ ), glucose ( $\text{g} \cdot 100\text{g}^{-1}$ ), saccharose ( $\text{g} \cdot 100\text{g}^{-1}$ ), conductivity ( $\text{mS} \cdot \text{cm}^{-1}$ ), invertase activity ( $\text{U} \cdot \text{kg}^{-1}$ ), diastase activity (Diastase number), pH and water activity analysis. Pollen analysis was not under the scope of this study. The methods used for analysis were based on those of the Association of the Official Analytical Chemists (AOAC, 1990) or those of the Harmonised Methods of the European Honey Commission and International Honey Commission (Bogdanov *et al.*, 1997a,b). Moisture was determined by use a honey automatic digital refractometer DR500 (Nguyen Anh Co, Ho Chi Minh City, Viet Nam). pH measurements were performed potentiometrically at 20°C with use of pH-meter Gryf 209L (Gryf HP, Prague, Czech Republic). Hydroxymethylfurfural (HMF) content was based on UV adsorbance at 550 nm (Spekol 1100, Analytic Jena – ZEISS Technology, Jena, Germany). Apparent reducing sugars (fructose, saccharose, glucose) were performed by HPLC method with spectrophotometer Spekol 1100. The enzyme activity is evaluated photometrically (Spekol 1100), by measuring the decomposition of the substrate p-nitrophenyl- $\alpha$ -D glucopyranoside into the product p-nitrophenol (which has a maximum absorbance at 400 nm). Electrical conductivity – was measured at 20°C in solutions of honey samples in deionized water with conductivity meter CDM 210 (Radiometer Analytical SAS, Lyon, France). Proline content was quantified with use of spectrophotometric method and absorbance determined at 510 nm (Spekol 1100). Water activity was determined by use of a water activity system Lab-Master-aw (Novasina, Pfaffikon, Switzerland).

### Statistical analyses

For statistical analysis, the Anova One-Way test, Kruskal-Wallis test, and Linear Model of Regression Analysis were performed with the computer program Statgraphics Plus version 5. 1 (AV Trading, UMEX, Dresden, Germany).

## RESULTS AND DISCUSSION

Value limits, as defined internationally by the European Honey Directive and the Codex Alimentarius, for honey of declared origin from regions, are amounts of not more than 50 milliequivalents of free acidity, 20% moisture,  $0.6 \text{ g} \cdot 100\text{g}^{-1}$  for general honey ash and  $40 \text{ mg} \cdot \text{kg}^{-1}$  for HMF. In addition, values of not less than  $8 \text{ U} \cdot \text{kg}^{-1}$  for invertase activity and 8 DN (diastase number) for diastase activity,  $60 \text{ g} \cdot 100\text{g}^{-1}$  for reducing sugars and  $180 \text{ mg} \cdot \text{kg}^{-1}$  for proline levels are prescribed. Some of these limits differ for honeydew honey, not less than  $45 \text{ g} \cdot 100\text{g}^{-1}$  of honey for reducing sugars and not more than  $1 \text{ g} \cdot 100\text{g}^{-1}$  for ash content (FAO, 2001; Bogdanov and Martin, 2002; EC, 2002).

Honey moisture content is used as an indicator of ageing and capacity to keep stable during storage (Bogdanov *et al.*, 1997a). The moisture content varied from 15.26% in the honeydew honey from west region to 17.65% in the honeydew honey from Slovakian west region (Table II). Moisture content is practically constant among Slovakian honeys, independently on region or kind of honey. When considering the Slovak Codex Alimentarius and (Slovak Codex Alimentarius, 2001) Slovak standard 1/2006 legislation (Statute of Slovak beekeeper union, 2006): 3 blossom, 2 blends and 3 honeydew honeys from the east Slovakia were outside of the legislation limits. Similarly, 3 blossom and 1 blend from the middle of Slovakia, and 2 blossom and 2 blends honeys from the west of Slovakia were out of standards.

Similarly results were found in the blossom honey ( $15.9 \pm 1.2\%$ ), blends honey ( $15.9 \pm 1.2\%$ ) and honeydew honey ( $15.6 \pm 1.7\%$ ) in the Czech Republic (Čelechovská and Vorlová, 2001). The moisture content in Slovak honey varied from 16.00 to 19.80% (Kačániová *et al.*, 2009). The moisture content of the Kenyan honey samples ranged from 15.60–21.20% (Muli *et al.*, 2007). Significant differences ( $P < 0.05$ ) were found in the moisture content between flowering periods, but not between production processes in the honey from Mexico (Ordóñez *et al.*, 2005). The moisture content of South Africa honey varied from ( $15.0 \pm 0.1\%$ ) to ( $25.1 \pm 0.1\%$ ) and sixty honey samples exceeded the permitted limit of 20% (FAO, 2001) and can be mainly explained by the premature extraction of these honeys (Meda *et al.*, 2005).

The methods for the determination of diastase and invertase activity were described (Bogdanov, 1997). Later, another formula was found for the diastase determination with the Phadebas method in honeys with low enzyme content (Persano-Oddo *et al.*, 1999). For the expression of invertase results, international

## II: Physicochemical characteristics of different honey types in different Slovakian regions

Type of honey	Slovakian region		
	East	Middle	West
<b>Moisture content (%)</b>			
blossom	17.4 ± 1.97	17.4 ± 1.89	16.2 ± 2.18
blends	16.1 ± 1.93	15.4 ± 2.04	17.7 ± 3.32
honeydew	17.3 ± 2.06	15.5 ± 0.71	15.3 ± 1.55
<b>HMF levels (mg.kg⁻¹)</b>			
blossom	27.60 ± 37.82	22.33 ± 10.17	63.00 ± 17.26
blends	21.83 ± 8.98	25.80 ± 7.95	30.17 ± 26.29
honeydew	28.33 ± 26.65	21.83 ± 8.98	24.20 ± 10.16
<b>Diastase activity (Diastase number DN)</b>			
blossom	21.01 ± 11.94	30.48 ± 8.86	21.01 ± 11.94
blends	27.09 ± 9.83	30.74 ± 7.54	30.99 ± 10.60
honeydew	23.03 ± 9.88	32.60 ± 7.92	36.67 ± 2.74
<b>Invertase activity (U.kg⁻¹)</b>			
blossom	121.73 ± 16.31	163.14 ± 36.75	149.79 ± 5.38
blends	159.04 ± 19.05	158.03 ± 18.48	143.36 ± 27.59
honeydew	147.55 ± 18.25	164.11 ± 22.74	149.63 ± 28.07
<b>Proline content (mg.kg⁻¹)</b>			
blossom	505.79 ± 153.20	531.72 ± 15.03	444.79 ± 35.35
blends	480.89 ± 93.91	458.37 ± 114.93	429.72 ± 48.61
honeydew	491.14 ± 84.91	464.04 ± 107.34	426.56 ± 47.22
<b>Fructose content (g.100g⁻¹)</b>			
blossom	39.60 ± 2.61	38.00 ± 4.56	39.00 ± 1.58
blends	37.50 ± 3.94	40.20 ± 2.28	37.33 ± 3.88
honeydew	36.33 ± 4.97	37.50 ± 3.94	39.60 ± 2.61
<b>Glucose content (g.100g⁻¹)</b>			
blossom	31.00 ± 2.24	29.33 ± 4.55	29.00 ± 5.43
blends	27.67 ± 5.58	27.20 ± 3.70	30.50 ± 4.85
honeydew	29.33 ± 6.50	27.67 ± 5.85	31.00 ± 2.24
<b>Saccharose content (g.100g⁻¹)</b>			
blossom	0.15 ± 0.00	0.27 ± 0.19	0.37 ± 0.33
blends	0.19 ± 0.10	0.37 ± 0.33	0.15 ± 0.00
honeydew	0.27 ± 0.19	0.19 ± 0.10	0.15 ± 0.00
<b>Electrical conductivity (mS.cm⁻¹)</b>			
blossom	29.48 ± 9.25	70.09 ± 12.56	30.15 ± 14.23
blends	80.25 ± 14.14	80.06 ± 17.61	97.06 ± 2.17
honeydew	86.75 ± 15.44	85.34 ± 18.20	97.24 ± 1.49
<b>pH value</b>			
blossom	4.26 ± 0.27	4.46 ± 0.29	4.06 ± 0.42
blends	4.68 ± 0.43	4.42 ± 0.24	4.41 ± 0.28
honeydew	4.80 ± 0.35	4.33 ± 0.14	4.37 ± 0.20
<b>Water activity content</b>			
blossom	0.55 ± 0.05	0.57 ± 0.04	0.56 ± 0.06
blends	0.55 ± 0.02	0.56 ± 0.07	0.56 ± 0.06
honeydew	0.57 ± 0.04	0.56 ± 0.05	0.56 ± 0.08

units ( $\text{U} \cdot \text{kg}^{-1}$ ) were proposed instead of Hadorn numbers (Von der Ohe *et al.*, 1999).

The most commonly monitored parameters for determining honey freshness include HMF levels and diastase and invertase activity (Persano-Oddo *et al.*, 1999; Bogdanov and Martin, 2002). According to our findings, HMF levels ranged from  $21.83 \text{ mg} \cdot \text{kg}^{-1}$  in the blends honey from east and honeydew honey from middle to  $63.00 \text{ mg} \cdot \text{kg}^{-1}$  in the blossom honey from west Slovakian region (Table II). The honeys from middle Slovakian region showed the lowest variation among regions at HMF. The diastase activity varied from 21.01 DN in the blossom honey from east and west region to 36.67 in the honeydew honey from west Slovakian region (Table II) and the invertase activity varied from  $121.73 \text{ U} \cdot \text{kg}^{-1}$  in the blossom honey from east region to  $164.11 \text{ U} \cdot \text{kg}^{-1}$  in the honeydew honey from middle Slovakian region (Table II). Values from west of Slovakia at invertase activity were very similar ( $143.63$ – $149.79 \text{ U} \cdot \text{kg}^{-1}$ ).

The different results of HMF were found by Če-lechovská and Vorlová (2001) in the Czech honey samples (blossom  $17.7 \pm 20.6 \text{ mg} \cdot \text{kg}^{-1}$ , blends  $20.4 \pm 17.5 \text{ mg} \cdot \text{kg}^{-1}$  and honeydew  $30.1 \pm 37.5 \text{ mg} \cdot \text{kg}^{-1}$ ). The results of diastase analysis in Italian honey samples varied in the different honey types ranged mostly from 0 to 35 DN. The honeydew honeys showed the highest values (20–50 DN) similarly to our results. The results of invertase determinations in Italian honey samples varied in the different honey types from less than 5 to more than  $200 \text{ U} \cdot \text{kg}^{-1}$ . Two honeydew honeys had the highest values (more than  $130 \text{ U} \cdot \text{kg}^{-1}$ ) and multifloral samples ranged from 50 to  $200 \text{ U} \cdot \text{kg}^{-1}$  (Persano-Oddo *et al.*, 1999).

Concerning invertase activity, honeydew honeys did not greatly differ from the compound ones. At the same time, the diastase activity was determined at all honeys as well as the ratio of both enzymes. In the invertase/diastase ratio, statistically significantly different ( $P < 0.05$ ) were only compound honeys ( $0.59 \pm 0.18$ ) from the type of honeydew honeys ( $0.82 \pm 0.27$ ) (Vorlová and Přidal, 2002).

27 samples (54%) of the honey were well inside the current Slovak standard (Slovak Codex Alimentarius, 2001). The multifloral honey sample with 6.5 DN could be qualified as a honey with low natural enzyme content (FAO, 2001). All the honey samples were in accordance to the limits for invertase and diastase activity.

The proline content varied from  $426.56 \text{ mg} \cdot \text{kg}^{-1}$  in the honeydew honey in the west region to  $531.79 \text{ mg} \cdot \text{kg}^{-1}$  in the blossom honey from the middle Slovakian region (Table II). West region was the most constant in the proline content of honey.

Some of the authors have reported that high values for proline are typical for honeydew honeys (Persano-Oddo *et al.*, 1999). In our study, the proline content of 2 honeydew honey samples were from 354.37 to  $662.58 \text{ mg} \cdot \text{kg}^{-1}$  but were not the highest values found. However, these values were higher than those of some groups of Moroccan honeydew honeys, which reportedly varied from 69 to  $556 \text{ mg} \cdot \text{kg}^{-1}$  (Diez

*et al.*, 2004). Authors such as Bogdanov (2002) believe that the majority of the proline comes from bee salivary secretions. Proline content has been shown to vary considerably between different honeys.

In sugar adulterated honeys the same chemical parameters such as enzyme activities, HMF content, ash content, electrical conductivity and proline content are decreased. These changes might indicate possible adulteration, if the normal variation of these parameters in different honeys is taken into account when interpreting the test for adulteration. Indeed, proline was suggested as a quality criterium for the honey with the respect to sugar adulteration. It was proposed that natural honeys should have proline content of more than  $180 \text{ mg} \cdot \text{kg}^{-1}$ . A lower proline content could mean that the honey has been adulterated with sugar. However, this value can be higher for certain honeys, as the proline content depends on the honey type. Also, it should be taken into account that some of these parameters as HMF and enzyme activity will change on heating and storage. Certain types of honeys such as citrus, acacia, rhododendron, honeydew and others have a higher natural content of the sugar (Bogdanov *et al.*, 2003).

The fructose content values were found from  $36.33 \text{ g} \cdot 100\text{g}^{-1}$  in the honeydew honey from the east region to  $40.20 \text{ g} \cdot 100\text{g}^{-1}$  in the blends honey from the middle Slovakian region (Table II), the glucose content values ranged from  $27.67 \text{ g} \cdot 100\text{g}^{-1}$  in the blends honey from the east region to  $31.00 \text{ g} \cdot 100\text{g}^{-1}$  in the blossom honey from the east region and honeydew honey from the west Slovakian region (Table II). The values of saccharose content were from  $0.15 \text{ g} \cdot 100\text{g}^{-1}$  in the blossom honey of East Slovakian region to  $0.37 \text{ g} \cdot 100\text{g}^{-1}$  of honey (Table II) in the blossom honey of West Slovakian region. Region did not show big influence on sugars content.

The values of glucose, fructose and saccharose were not within the limits listed in the Slovak standard (Slovak Codex Alimentarius, 2001) and Codex Alimentarius (FAO, 2001). These values, however, seemed to be higher than those for commercial honey from Australia, China, Egypt, Germany, Morocco, Pakistan, Qatar, USA, Italy and Yemen (Al-Jedah *et al.*, 2003).

Careless handling of honey can reduce its quality. Among the factors that most influence quality are – high temperatures, length of storage and moisture content, greater than 21%. They lead to fermentation, high levels of hydroxymethylfurfural (Sancho *et al.*, 1992) loss of enzymatic activity, changes in flavour, darkening (Ordóñez *et al.*, 2005) and microbial growth (Schocken-Iturrino *et al.*, 1999).

The method for the determination of electrical conductivity is described in Bogdanov *et al.* (1997a,b). According to Codex Alimentarius honeys values (FAO, 2001) are expressed in  $\text{mS} \cdot \text{cm}^{-1}$  at  $20^\circ\text{C}$ , while nowadays the international reference measurements should be carried out at  $25^\circ\text{C}$ . This contradiction needs to be resolved.

The conductivity varied from  $29.48 \text{ mS} \cdot \text{cm}^{-1}$  in the blossom honey from the east region to

97.24 mS.cm<sup>-1</sup> in the honeydew honey from the west Slovakian region (Table II). According to Slovak standards (Slovak Codex Alimentarius, 2001) and Codex Alimentarius (FAO, 2001) maximal values for blossom honeys (except of chestnut honey) are introduced for differentiation between honeydew and blossom honeys (Bogdanov *et al.*, 2004).

The methods for the determination of free acidity by titration to pH 8.3 or to an equivalence point have a poor reproducibility (Bogdanov *et al.*, 1997a; Bogdanov *et al.*, 1997b), due to lactone hydrolysis during titration. The reproducibility of the measurement of total activity (free acidity + lactones) is slightly better.

The pH value varied from 4.06 in the blossom honey to 4.80 in the honeydew honey (Table II). Published reports indicate that pH should be between 3.2 and 4.5 (Meda *et al.*, 2005). The mean values, however, only indicated that some of the honey samples were outside of this range (4.68; 4.80). Some of the honeys, such as chestnut and fir honey have been shown to have high pH values viz. 5–6 and 4.6–5.9, respectively (Meda *et al.*, 2005).

The water activity content varied from 0.55 in the blossom and blend honey to 0.57 in the honeydew and blossom honey (Table II). Values of water activity were stable among types of honey and regions too. The water activity of honey depends mainly on the glucose content. During crystallisation, glucose starts to crystallise first. Fructose has a higher solubility and stays in solution for a longer time. All the five hydroxyl groups of glucose interact with water molecules. The difference between the water activities of the different honey types is the result of its diverse sugar compositions. Values of water activity between 0.53 and 0.63 are absolutely safe for other foods as concerns the risk of microbiological spoilage (Gleiter and Horn, 2006).

Statistical analysis was done to determine differences in quality between honeys from the three honey types. In total statistical results showed, that significant differences ( $P \leq 0.05$ ) were found between HMF and fructose, glucose and saccharose and conductivity and pH. Significant differences ( $P \leq 0.01$ ) were found between fructose and glucose and significant differences ( $P \leq 0.001$ ) were found between HMF and conductivity. No significant differences ( $P \geq 0.05$ ) were found between another parameters (Table III). Significant differences ( $P \leq 0.05$ ) by the types

of honey were found between HMF and significant differences ( $P \leq 0.001$ ) were found between conductivity (Table IV). Significant differences ( $P \leq 0.05$ ) by the regions of honey were found between HMF and invertase and significant differences ( $P \leq 0.01$ ) were found between conductivity (Table V).

A significant positive correlation was detected between moisture-HMF ( $P \leq 0.05$ ) in Slovak honey samples. No significant correlations were detected between moisture and other physicochemical indicators. A significant positive correlation was detected between HMF-moisture ( $P \leq 0.05$ ) in Slovak honey samples. No significant correlations were detected between HMF and other physicochemical indicators (Kačániová *et al.*, 2009).

Honey produced in the Slovakia, has physicochemical properties that are desirable in the international market. Region of Slovakia did not show big influence on the honey composition. Harvesting, bottling and storing of the honey have a very little input of technology. Apiculture in Slovakia needs to be focused on increasing of production as much as preserving quality through proper handling. Therefore, a study was carried out to evaluate the physicochemical quality of honey from *Apis mellifera* during one production cycle including three honey types and the regions in the production process.

### III: Correlation coefficients values of different honey types

Parameter	P-value
Water	0.3731
HMF	0.0447*
Proline	0.8757
Fructose	0.8506
Glucose	0.8153
Saccharose	0.7880
Conductivity	0.0000***
Diastase	0.9557
Invertase	0.4885
pH	0.1280
a <sub>w</sub>	0.9083

\*\*\*P ≤ 0.001; \*\*P ≤ 0.01; \*P ≤ 0.05, -P ≥ 0.05

## SÚHRN

### Fyzikálno-chemické vlastnosti medu z rôznych regiónov Slovenska

Cieľom práce bolo zistíť fyzikálno-chemické vlastnosti slovenského medu a porovnať ich s uvedenými štandardmi (Codex Alimentarius, Európska smernica medu, Slovenský Potravinový kódex a Slovenská norma pre med). Ďalej sme sledovali korelácie medzi jednotlivými ukazovateľmi medu. Fyzikálno-chemické parametre medu boli zistované v súlade s pravidlami Medzinárodnej Komisie pre med IHC. Vzorky medu (n=50) boli kvetové, zmiešané a medovicové, pôvodom z východného, stredného a západného Slovenska. Fyzikálno-chemické sledovanie zahŕňalo stanovenie vlhkosti, redukujúcich cukrov, prolinu, hydroxymetylurfuralu, konduktivity, diastázovej aktivity, pH a vodnej aktivity v súlade s nariadeniami Európskej Komisie pre med EHC. Obsah vody v mede sa

## IV: Correlation coefficients values of dependences physicochemical characteristics (Linear model of simple regression)

	Moisture	HMF	Proline	Fructose	Glucose	Saccharose	Conductivity	Diastase	Invertase	pH	aw
Moisture	-	-0.053	0.060 <sup>-</sup>	-0.138 <sup>-</sup>	0.155 <sup>-</sup>	-0.003 <sup>-</sup>	-0.051 <sup>-</sup>	0.028 <sup>-</sup>	-0.203 <sup>-</sup>	-0.011 <sup>-</sup>	-0.096 <sup>-</sup>
HMF		-	-0.034 <sup>-</sup>	0.289 <sup>*</sup>	0.232 <sup>-</sup>	0.127 <sup>-</sup>	-0.504 <sup>***</sup>	0.059 <sup>-</sup>	-0.243 <sup>-</sup>	-0.154 <sup>-</sup>	0.029 <sup>-</sup>
Proline			-	-0.053 <sup>-</sup>	-0.172 <sup>-</sup>	0.033 <sup>-</sup>	0.013 <sup>-</sup>	-0.111 <sup>-</sup>	0.124 <sup>-</sup>	0.150 <sup>-</sup>	0.110 <sup>-</sup>
Fructose				-	0.395 <sup>**</sup>	-0.106 <sup>-</sup>	-0.180 <sup>-</sup>	0.136 <sup>-</sup>	0.164 <sup>-</sup>	-0.148 <sup>-</sup>	-0.076 <sup>-</sup>
Glucose					-	-0.285 <sup>*</sup>	-0.047 <sup>-</sup>	0.014 <sup>-</sup>	-0.179 <sup>-</sup>	-0.075 <sup>-</sup>	-0.069 <sup>-</sup>
Saccharose						-	-0.114 <sup>-</sup>	0.038 <sup>-</sup>	0.071 <sup>-</sup>	0.083 <sup>-</sup>	0.157 <sup>-</sup>
Conductivity							-	0.059 <sup>-</sup>	0.147 <sup>-</sup>	0.356 <sup>*</sup>	0.197 <sup>-</sup>
Diastase								-	0.034 <sup>-</sup>	-0.230 <sup>-</sup>	-0.167 <sup>-</sup>
Invertase									-	-0.047 <sup>-</sup>	0.076 <sup>-</sup>
pH										-	0.104 <sup>-</sup>
$a_w$											-

\*\*\*P ≤ 0.001; \*\*P ≤ 0.01; \*P ≤ 0.05, -P ≥ 0.05

## V: Correlation coefficients values of different regions

Blossom	P-value	Blends	P-value	Honeydew	P-value
Water	0.4368	Water	0.1874	Water	0.2391
HMF	0.0262*	HMF	0.7543	HMF	0.8712
Proline	0.9295	Proline	0.5966	Proline	0.4971
Fructose	0.8714	Fructose	0.2758	Fructose	0.5919
Glucose	0.8747	Glucose	0.4147	Glucose	0.8032
Saccharose	0.3037	Saccharose	0.2035	Saccharose	0.3525
Conductivity	0.0045**	Conductivity	0.0422*	Conductivity	0.3357
Diastase	0.0881	Diastase	0.8302	Diastase	0.1282
Invertase	0.0379*	Invertase	0.1874	Invertase	0.6269
pH	0.3016	pH	0.5043	pH	0.0671
$a_w$	0.4399	$a_w$	0.8801	$a_w$	0.8219

\*\*\*P ≤ 0.001; \*\*P ≤ 0.01; \*P ≤ 0.05, -P ≥ 0.05

pohyboval od 15,26 do 17,65 %, obsah HMF sa pohyboval od 21,83 do 63,00 mg.kg<sup>-1</sup>, aktivita diastázy bola od 21,01 do 36,67 DN, aktivita invertázy sa pohybovala od 121,73 do 164,11 U.kg<sup>-1</sup>, obsah prolínu sa pohyboval od 426,56 do 531,79 mg.kg<sup>-1</sup>, obsah fruktózy bol od 6,33 do 40,20 g.100g<sup>-1</sup>, obsah glukózy sa pohyboval od 27,67 do 31,00 g.100g<sup>-1</sup>, obsah sacharózy bol od 0,15 do 0,37 g.100g<sup>-1</sup>, konduktivita sa pohybovala od 29,48 do 97,24 mS.cm<sup>-1</sup>, pH sa pohybovalo od 4,06 do 4,80 a hodnota vodnej aktivity sa pohybovala v rozmedzí od 0,55 do 0,57. Preukazné rozdiely (P ≤ 0,05) boli zistené medzi HMF a fruktózou, glukózou a sacharózou, konduktivitou a pH; vysoko preukazné rozdiely (P ≤ 0,01) medzi fruktózou a glukózou a vysoko preukázané rozdiely (P ≤ 0,001) medzi HMF a konduktivitou. Medzi kvetovými a medovicovými medmi boli zistené štatistikovo významné rozdiely u HMF (P ≤ 0,05) a u konduktivity medzi medovicovými a kvetovými medmi (P ≤ 0,001). Medzi regiónmi boli zistené štatistikovo významné rozdiely medzi HMF a invertázou (P ≤ 0,05) a konduktivitou (P ≤ 0,01).

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