

The influence of geographical origin on honey composition studied by Polish and Slovak honeys

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Abstract: Honey composition is mainly affected by botanical origin, however geographical factors as well as bee-keeping practice and storage conditions can also influence its quality. The aim of the study was to determine the impact of geographical origin on physicochemical quality and biological activity of honey. For this reason Polish and Slovak varietal honeys, including per each country: 10 multifloral, 5 tilia, 5 rape, 5 acacia and 5 forest were compared according to their physicochemical parameters (free acidity, pH, electrical conductivity, moisture content, and colour intensity), sugar profile, diastase activity, as well as antioxidant activity (DPPH and FRAP tests, as well as photochemiluminescence method). Moreover, total phenolics compounds and flavonoids content were determined. The most significant differences ($P < 0.05$) between Polish and Slovak counterparts were found for tilia while the lowest for rape honeys. The impact of geographical origin on overall quality of honey was proved by PCA statistical tool.

Keywords: antioxidant activity; geographical origin; honey; HPLC; physicochemical parameters

Honey is known as antioxidant (DŻUGAN *et al.* 2018) and antimicrobial (KAČÁNIOVÁ *et al.* 2012) natural remedy. However, the chemical composition of honey strongly depends on the kind of nectar flow. In turn, biochemical profile of nectar is qualitatively and quantitatively influenced by genetics and physiology of the source plant, environmental factors (climatic conditions), soil characteristics and typology of pollinators (GISMONDI *et al.* 2018). Due to, it can be expected that honey quality depends on both botanical and geographical origin (KAŠKONIENĚ & VENSKUTONIS 2010). It was proved that the composition of the plants essential oils is geographically dependent, even for the same plant species, which suggests that honey with the same floral origin but from different locations may have a different composition (KAŠKONIENĚ & VENSKU-

TONIS 2010). Next to botanical and geographical origin, the quality of honey can be influenced by other factors, such as the strength and vitality of the bee colony, the manner of collecting and confectioning honey by beekeepers, as well as the storage of honey by the consumer (SOARES *et al.* 2017).

The aim of the study was to determine the influence of geographical origin on honey physicochemical quality and biological activity based on the comparison of Polish and Slovak varietal honeys.

MATERIAL AND METHODS

For comparison 60 varietal honey samples including 10 multifloral, 5 tilia, 5 rape, 5 acacia and 5 forest per each country were collected in 2015 directly

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from local beekeepers operating in South-Eastern part of Poland and North-Eastern part of Slovakia, respectively. The varieties of honey were determined by the beekeepers based on availability of floral sources and regarding to hive location. Until the time of analysis, all samples were stored at room temperature in the laboratory but no more than 6 months.

Physicochemical properties. Titratable acidity (TA), pH, electrical conductivity and moisture content were determined according to IHC (2009). Colour intensity was determined for 50% (g/l) aqueous solution of honey (homogenized and centrifuged at 14000 rpm for 5 min) and absorbance measured at 450 nm, using a spectrophotometer Biomate 3 (Thermo, USA). Colour intensity was expressed as mAU (PONTIS *et al.* 2014).

Sugar profile. Sugar content was determined by high-performance liquid chromatograph (Thermo Dionex Ultimate 3000; Thermo Fisher Scientific, USA) equipped with corona discharge detector (Corona Veo RS; ESA Chelmsford, USA) and Shodex Asahipak NH2P-504E (4.6 × 250 mm) chromatography column. Sample volume of 10 µl (2% g/l solution in ethanol) was injected. The separation was conducted at a temperature of 55°C with the mobile phase acetonitrile:water 78:22 (v/v), at a flow rate of 1 ml/min. The content of tested carbohydrates (fructose, glucose and sucrose) was calculated based on the external standard curve and expressed as g per 100 g of honey.

Diastatic activity. Determination of the diastatic activity of honey was performed using Phadebas Honey Diastase Test© (Magle AB Lund, Sweden) according to procedure added to the test kit. The results were expressed as DN based on conversion table appended to the test.

Bioactive compounds analysis

DPPH radical scavenging assay. The antioxidant activity of honey samples was assessed using DPPH assay according to DŽUGAN *et al.* (2018). The scavenging activity was expressed as % of DPPH radical inhibition.

Ferric reducing antioxidant power assay (FRAP). The FRAP assay was carried out as according to BERTONCELJ *et al.* (2007). For the calibration curves was used Trolox (0.1 mM) in the range 15–200 nmol and the results were expressed as µmol of Trolox equivalents (TE) per kg of honey (µmol TE/kg of honey).

Photochemiluminescence method (PCL). Antioxidant activity tested by PCL method using the Photochem device (Jena, Germany) according to WESOŁOWSKA and DŽUGAN (2017) allowed to determine water (ACW) and fat (ACL) soluble antioxidant fractions. Measurements were performed using reagent kits provided by the manufacturer according to the attached instruction. Results were expressed as mmol of Ascorbic acid (AA) per 1 kg of honey for ACW and µmol of Trolox per 1 kg of honey for ACL.

Total phenolic compounds (TPC) content. The Folin–Ciocalteu method modified by BERETTA *et al.* (2005) was used to determine total phenolic compounds content in honey. Gallic acid (0–200 mg/ml) was used for calibration. Total phenolic compounds content was expressed as mg of gallic acid equivalent per kg of honey (mg of GAE/kg) as proposed PILJAC-ŽEGARAC *et al.* (2009).

Total flavonoids (TFC) content. The total flavone and flavonol contents of the honey samples were determined using AlCl₃ according to WIECZOREK *et al.* (2014). A standard curve of quercetin was prepared within a concentration range of 3–40 µg/ml and the results were expressed as mg quercetin per kg of honey (mg of QE/kg).

Statistical analysis. Data were reported as mean ± standard deviation of at least three replications. Statistical analysis of the results was performed with the software Statistica 13.1. (StatSoft Inc., USA). One-way ANOVA followed by Tukey's HSD test was used to investigate the differences between honey of the same varieties originating from different countries and $P < 0.05$ was accepted as significant. Correlations among analysed parameters were calculated by Pearson correlation coefficients (r) at a significance level of 95% ($P < 0.05$). Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method.

RESULTS AND DISCUSSION

Honey quality evaluation regarding UE standards. The Polish and Slovak honeys were controlled according to the applicable UE legal regulation (Council Directive 2001) (Table 1). All of tested honey samples fulfilled the legal regulations limits. In Slovak samples, the lower conductivity as compared to Polish counterparts was observed, however only in tilia and acacia honeys the differences were significant ($P < 0.05$) (Table 1). ACQUARONE *et al.* (2007) suggest

Table 1. Physicochemical quality and biological activity of tested Polish (PL) and Slovak (SK) honey samples

	Multifloral		Tilia		Forest		Rape		Acacia	
	PL	SK	PL	SK	PL	SK	PL	SK	PL	SK
	(n = 10)									
Moisture (%)	18.65 ± 1.34	18.53 ± 0.80	17.76 ± 0.57	18.35 ± 0.52	17.40 ± 1.12	17.98 ± 0.38	17.86 ± 1.14	17.45 ± 0.38	17.73 ± 0.51	17.86 ± 0.21
Conductivity (mS/cm)	0.35 ± 0.08	0.21 ± 0.12	0.53 ± 0.22*	0.23 ± 0.09*	0.82 ± 0.25	0.37 ± 0.23	0.23 ± 0.15	0.16 ± 0.08	0.42 ± 0.27*	0.20 ± 0.09*
Free acidity (mEq/kg)	37.0 ± 7.3*	23.3 ± 11.8*	34.2 ± 7.0*	21.6 ± 11.6*	33.6 ± 5.2	28.1 ± 8.5	18.6 ± 2.2	13.6 ± 2.8	25.6 ± 6.0	16.1 ± 7.1
Diastase (DN)	22.56 ± 5.35	13.14 ± 4.87	31.99 ± 9.60*	19.08 ± 6.93*	23.06 ± 6.05	21.09 ± 11.63	17.61 ± 6.80	12.38 ± 1.93	16.45 ± 1.19	14.15 ± 5.11
pH	3.57 ± 0.14	3.68 ± 0.13	3.81 ± 0.25	3.90 ± 0.18	4.16 ± 0.24	4.07 ± 0.14	3.88 ± 0.11	3.61 ± 0.06	3.79 ± 0.29	3.71 ± 0.23
Color intensity (mAU)	699 ± 259*	308 ± 186*	820 ± 241*	306 ± 74*	1098 ± 295	823 ± 173	427 ± 162	197 ± 37	422 ± 168	221 ± 220
Fructose (%)	39.23 ± 3.08	38.66 ± 2.52	36.89 ± 3.26	38.15 ± 2.35	34.67 ± 0.31	37.83 ± 0.68	36.35 ± 2.28	36.39 ± 1.44	37.64 ± 1.60	37.34 ± 1.66
Glucose (%)	31.82 ± 1.78	27.78 ± 3.32	28.43 ± 1.37	28.21 ± 1.81	29.10 ± 1.71	26.78 ± 1.83	32.92 ± 2.80	29.69 ± 1.57	31.75 ± 1.77	26.14 ± 0.88
Sucrose (%)	4.40 ± 1.95	4.47 ± 2.15	6.12 ± 1.32	4.68 ± 1.22	6.29 ± 1.07	5.06 ± 0.61	5.06 ± 0.54	4.61 ± 0.23	3.53 ± 0.76	5.27 ± 1.50
DPPH (% inhibition)	36.12 ± 12.67	22.24 ± 10.19	40.71 ± 13.30*	18.84 ± 1.75*	60.52 ± 3.83	46.53 ± 13.69	21.21 ± 3.76	11.76 ± 2.40	24.80 ± 7.98	14.47 ± 4.27
FRAP (mmol TE/kg)	1.32 ± 0.53	1.11 ± 0.51	0.95 ± 0.22	1.17 ± 0.33	2.32 ± 1.04	2.84 ± 0.55	0.68 ± 0.16	0.59 ± 0.06	1.59 ± 0.75	0.64 ± 0.11
ACW (mmol AA/kg)	16.10 ± 5.27	13.73 ± 5.53	12.62 ± 2.75	12.01 ± 2.16	19.09 ± 3.30	18.80 ± 4.61	9.93 ± 3.47	7.98 ± 1.03	15.77 ± 4.19	8.17 ± 1.28
ACL (mmol Trolox/kg)	2.19 ± 1.19	1.35 ± 0.58	1.48 ± 0.59	1.51 ± 0.54	2.34 ± 0.42	2.81 ± 0.58	0.67 ± 0.15	0.59 ± 0.16	1.73 ± 0.61	0.64 ± 0.13
TPC (g GAE/kg)	0.46 ± 0.11	0.34 ± 0.15	0.38 ± 0.04	0.35 ± 0.11	0.60 ± 0.24	0.68 ± 0.20	0.25 ± 0.05	0.21 ± 0.04	0.47 ± 0.011*	0.20 ± 0.05*
Flavonoids (mg QE/kg)	4.96 ± 2.37*	2.92 ± 1.23*	4.99 ± 1.90*	2.57 ± 1.18*	6.68 ± 1.54*	5.39 ± 0.95*	3.24 ± 0.87*	2.16 ± 0.46*	3.15 ± 1.47*	1.37 ± 0.70*

*significant differences ($P < 0.05$) between PL and SK honey within the counterparts

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that the high ash content of honey (and thus high conductivity) is related to geographical origin and is likely due to the high mineral content of the soil. Values recorded in present study were comparable with other authors findings for Polish (PRZYBYŁOWSKI & WILCZYŃSKA 2001; POPEK 2002) and Slovak (KASPEROVÁ *et al.* 2012) honeys. The pH measured for Polish and Slovak honey samples were comparable ($P > 0.05$) and were in agreement with SZCZĘSNA *et al.* (2011) findings for Polish honeys, while KASPEROVÁ *et al.* (2012) indicated slightly higher values of pH for Slovak honeys. Honey pH depends on both the ionized acids as well as mineral elements and among other properties influences microorganisms development, enzymatic activity, and texture (CAVIA *et al.* 2007). The free acidity of Polish samples was higher as compared to Slovak ones and in the case of multifloral and tilia honeys the differences were significant ($P < 0.05$) (Table 1). Present study results were comparable to other authors findings for Polish (PRZYBYŁOWSKI & WILCZYŃSKA 2001; POPEK 2002; MAJEWSKA *et al.* 2015) and Slovak (KASPEROVÁ *et al.* 2012) honeys. Diastase activity expressed as diastase number (DN) which is approved marker of honey quality, used in thermal processing evaluation, was detected in Polish honey samples in the higher levels as compared to Slovak counterparts (Table 1). The most significant difference ($P < 0.05$) between Polish and Slovak tilia honey was observed. The diastase originate not only from bees (the hypopharyngeal glands secretions), but also it has a vegetal origin (nectar or honeydew) (JUAN-BORRAS *et al.* 2014). The colour intensity of analysed honey samples measured spectrophotometrically (Abs_{450}) indicated differences in Polish and Slovak counterparts, significant for multifloral and tilia honeys ($P < 0.05$). However, for both countries the lightest colour for rape honey, while as the darkest for forest honeys were found. The colour of honey is an important parameter, which reflects the source of the nectar flow (PONTIS *et al.* 2014). The differences between colour intensity in Polish and Slovak honeys can be due to the geographical origin but also beekeeping practice and storage conditions. The sugar profile of tested honey samples from both countries was similar ($P > 0.05$). According to the Council Directive EU (2001) a good quality honey should contain no more than 5 g per 100 g sucrose and at least 60 g per 100 g of reducing sugars. The sum of reducing sugars in Polish and Slovak honey samples was compared to the values reported by other authors (ZIELIŃSKA *et al.* 2014).

The sucrose contents of the tested honey samples did not exceed the established limits and were comparable with other authors findings (CAVIA *et al.* 2007). According to JUAN-BORRAS *et al.* (2014) even though honey contains an active sucrose splitting enzyme (sucrase and glucosidase), the sucrose level in honey never reaches zero. In all tested honey varieties fructose content was higher than glucose (Table 1). According to ZAFAR *et al.* (2008) in the honey of good quality, the fructose content should exceed that of glucose.

Bioactive compounds analysis. Antioxidant activity measured by DPPH test for Polish samples was slightly higher as compared to Slovak counterparts, but only in the case of Polish tilia honey the difference was significant ($P < 0.05$). Obtained results were comparable to the study of WILCZYŃSKA (2010), for Polish and to KACANIOVA *et al.* (2011) for Slovak honeys. Reducing antioxidant power measured by FRAP test was comparable for honeys originated from both countries (Table 1). The highest reducing antioxidant power was tested in forest honey regardless of the geographical origin. Obtained results were in agreement with other authors findings (GHELDOLF & ENGESETH 2002; DŽUGAN *et al.* 2018). PCL method, less commonly used, allowed to differentiate antioxidants in honey regards to their water solubility into hydrophilic and hydrophobic fractions. PCL analysis confirmed that, regardless of the geographical origin, only minor fraction of honey antioxidants (about 6–12%) exhibit hydrophobic properties. In all tested samples, water-soluble fraction of antioxidants was on average by 5–20 fold higher than the water-insoluble fraction (ACW/ACL ratio from 6.5 to 14.5). Presented results are in agreement with WESOŁOWSKA and DŽUGAN (2017) earlier study for Polish honeys.

Since antioxidant and antiradical properties of honey have been mainly attributed to the presence of phenolic compounds (ZÜBEYİR *et al.* 2017), such components were also analysed in tested honeys. In general, total phenolics content (TPC) in tested honeys from both countries were comparable ($P > 0.05$), excluding acacia honey where significantly higher ($P < 0.05$) level of this compounds for Polish samples was determined. Obtained results were in agreement with other authors findings (BERTONCELJ *et al.* 2007; WILCZYŃSKA 2010; MELLEEN *et al.* 2015). In the group of phenolic compounds, next to phenolic acids, flavonoids as variety dependent honey components are classified (LIBERATO *et al.*

2011; PONTIS *et al.* 2014). The total flavonoid content (TFC) determined spectrophotometrically was positively correlated with total phenolic compounds ($r = 0.75$) and constitutes about 1.0–1.3% of total phenolics. All of tested Polish samples were characterized by significantly higher level of flavonoids ($P < 0.05$) in comparison to their Slovak counterparts. Similar flavonoids content in Polish honey samples found WIECZOREK *et al.* (2014).

Statistical analysis. High positive Pearson's correlation coefficients (r) between colour intensity and antioxidant activity measured by different methods was found ($r = 0.581$ – 0.840). Moreover, significantly strong positive correlation was calculated between colour intensity and TPC and TFC ($r = 0.703$ and 0.919) indicating that these compounds are responsible for colour of honey and its antioxidant activity, which was proved before by other authors (DŽUGAN *et al.* 2018; ANAND *et al.* 2018).

In order to study the influence of tested physicochemical parameters and bioactive compounds on honey quality, a PCA study was carried out. In the PCA analysis, PC1 was mainly related with antioxidant activity and physicochemical parameters and PC2 was positively associated with fructose, glucose and hydrophilic fraction of antioxidants (Figure 1). Regarding the influence of the parameters, it can be observed that antioxidant activity tested by different methods (FRAP, DPPH, ACW, ACL, TPC, and TFC) as well as colour intensity influence significantly the

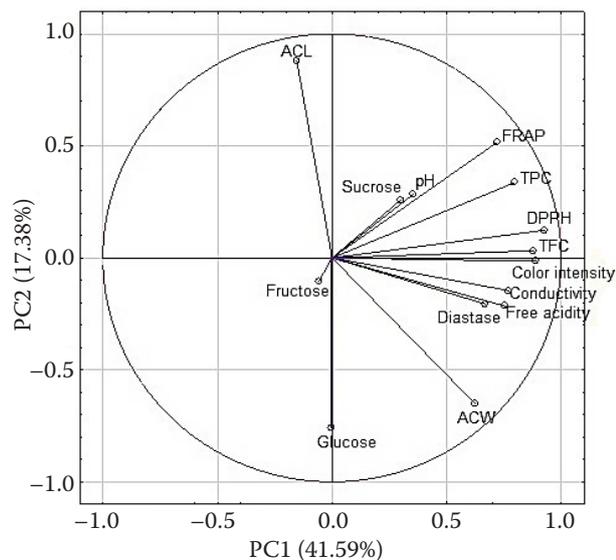


Figure 1. Principal Component Analysis (PCA) – biplot of scores and loadings of data obtained from physicochemical and bioactive compounds determinations, distribution of variables

projection, while sugar profile (sucrose, fructose and glucose) as well as pH show no significant influence on the overall quality of honey. Such results are in agreement with OROIAN *et al.* (2018).

Moreover, the PCA correlation plots for individual samples show the clustering of honey into two main groups: Polish and Slovak samples (Figure 2). In general, the differences obtained among the samples for all tested parameters clearly show that the geographical origin of honey significantly influence its overall quality. It is in agreement with other authors evidences that geographical origin influences honey physicochemical parameters (SOHAIMY *et al.* 2015), mineral composition (LASIĆ *et al.* 2018) and antioxidant activity (MELLEN *et al.* 2015; SOARES *et al.* 2017).

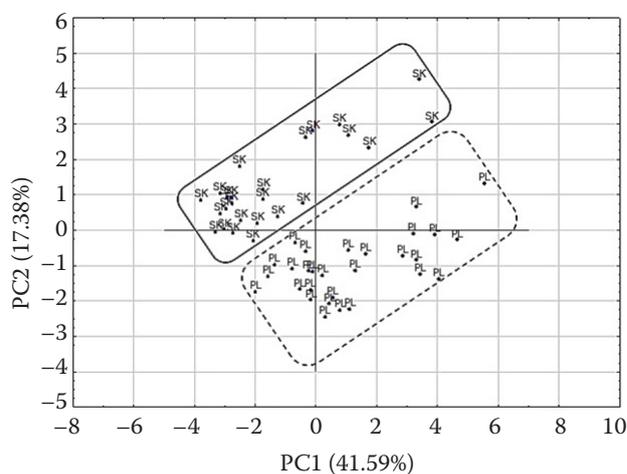


Figure 2. Principal Component Analysis (PCA) – biplot of scores and loadings of data obtained from physicochemical and bioactive compounds determinations, distribution of honey samples from Poland (PL) and Slovakia (SK)

CONCLUSIONS

Results indicated that all tested honey samples from both countries Poland and Slovakia fulfilled the European standards according to their physicochemical quality. In the tested collection of honey samples, the difference in the content of biologically active compounds has been confirmed. Honey of the same variety from different countries showed a variation in terms of antioxidant activity, diastatic activity and physicochemical properties. Among tested honey varieties, tilia honey from both countries differed the most in antioxidant and physicochemical parameters, while Polish and Slovak rape honey exhibited the most similar properties. Based on PCA

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analysis the impact of geographical origin of honey on its properties was confirmed.

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