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PHYSICO-CHEMICAL PROPERTIES AND BIOACTIVE COMPOUNDS IN SLOVAK HONEYS

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ABSTRACT

Honey quality and authenticity are key factors affecting consumer trust and market value. However, differences between honey from small-scale apiaries and commercial production remain insufficiently characterised. This study evaluated selected physico-chemical parameters of Slovak honey samples to assess their quality and authenticity. Twenty-five apiary samples and six commercial samples (from retail and chain stores) were analysed. Basic analyses included water content (by refractometry), free acidity (FA) and pH (by potentiometric titration), electrical conductivity (EC, by conductometry), and hydroxymethylfurfural content (HMF, by HPLC). Special analyses focused on protein determination, particularly royal jelly proteins (RJPs) and apalbumin 1 (APA1, the major royal jelly protein), as well as antioxidant activity (AA, by DPPH• method) and total phenolic content (TPC, by spectrophotometry). Apiary samples were evaluated based on botanical and geographical origin. *Brassica napus* and *Robinia pseudoacacia* honeys exhibited significantly lower pH, EC, AA, TPC, and RJPs, which appears to be a natural characteristic of these honey types. These honeys originated from apiaries mainly in lowland areas (altitudes up to 300 m). When comparing apiary and commercial samples, apiary honeys contained lower HMF levels. Significant differences were also observed in protein content, particularly RJPs, which were higher in apiary samples. This suggests that apiary honey may contain a broader spectrum of biologically active substances. A more detailed characterization of Slovak honey in terms of sensory, physico-chemical, and biological properties could enhance its potential applications as a functional food in the future.

Keywords: Acidity, Antioxidant activity, Apalbumin 1, Bee product, Hydroxymethylfurfural

INTRODUCTION

Despite its relatively small size (49,000 square km), Slovakia exhibits a wide variety of honey types. The diversity of bee pasture is notable given the country's varied topography, which includes lowlands, hills, and mountains. In the Slovak territory, 2,976 species of vascular plants are present, excluding subspecies and *Pteridophyta*. This flora includes approximately 2,420 species of herbaceous plants, 304 species of lianas, semi-shrubs, and shrubs, and 166 species of trees (**Dostál & Červenka, 1991, 1992**). The bee populations in Slovakia predominantly consist of *Apis mellifera carnica*, with noticeable admixtures of *A. m. ligustica*, *A. m. macedonica*, and Buckfast bee (**Bauer *et al.*, 2017**). Among the main European monofloral honeys, described by **Oddo *et al.* (2004)**, there are honeys derived from rapeseed/canola (*Brassica* spp.), false acacia/black locust (*Robinia pseudoacacia* L.), sunflower (*Helianthus annuus* L.), lime tree (*Tilia* spp.) and honeydew. Additionally, less commonly, honeys from heather (*Calluna vulgaris* L.) and chestnut (*Castanea sativa* L.) are also produced. Slovakia has a considerable number of forests, which provide both nectar and honeydew for bees. Honeys from Slovak forests originate from blossom and/or honeydew. Honeys produced in Slovak forests can originate from blossom sources, honeydew, or a combination of both, depending on the available bee pasture. Consequently, forest honeys can be classified as blossom, blended (nectar-honeydew), or pure honeydew honeys. The differences in physico-chemical properties primarily depend on the type of honey. Apart from botanical origin, several other factors significantly influence the physico-chemical properties of honey including geographical origin, the intensity of nectar flow, climatic conditions, bee species, beekeeping practices, handling and packing procedures, as well as the time and conditions of storage (**Machado De-Melo *et al.*, 2018**; **Moo-Huchin *et al.*, 2015**; **Ramírez-Miranda *et al.*, 2021**; **Thrasyvoulou *et al.*, 2018**).

Biological activity of honey depends on present chemical substances - mainly biologically active substances. Honey consists of water (12-20%), saccharides (80%), organic acids (0.57%), enzymes and other chemicals in minor amounts, e.g. other special bee proteins, amino acids (mainly proline), vitamins (mainly vitamins B_1, B_2 and C), mineral substances (mainly potassium), aromatic and phenolic substances (**Al-Farsi *et al.*, 2018**; **Almasaudi, 2021**; **Bíliková *et al.*, 2015**; **da Silva *et al.*, 2016**; **Tomczyk *et al.*, 2023**). Water content is related to ripeness as well as storability of honey. During the creation of honey from the nectar, water decreases by 40-70% in weight (**Ruiz-Argueso & Rodriguez-Navarro, 1975**). High water content can lead to a growth of yeasts and moulds, causing fermentation, flavour loss and low shelf life (**Al-Farsi *et al.*, 2018**). Honey is acidic in terms of pH values, but there is no legislative limit for acidity. Acidity is caused by the presence of organic acids and is a characteristic feature of honey contributing to its antibacterial activity because most microorganisms optimally grow at a neutral pH range of 6.5 to 7.5. Dominant acid in the honey is gluconic acid (0.5% w/v) (**Almasaudi, 2021**). Along with this dominant acid, honey contains small amounts of other acids, which originate from plants or are results of microbial activities during the transformation of nectar into honey, such as lactic or acetic acid (**Ruiz-Argueso & Rodriguez-Navarro, 1975**). The spectrum of organic acids is wide and can act as a fingerprint for honey, because it is used to discriminate the honeys according to their botanical and/or geographical origin (**da Silva *et al.*, 2016**; **Machado De-Melo *et al.*, 2018**). General limit for free acidity (FA) is 50 meq/kg (**Council Directive 2001/110/EC, 2001**). FA differs according to honey type (**Oddo *et al.*, 2004**; **Živkov Baloš *et al.*, 2018**). In general, blossom honey has lower FA compared to honeydew honey. However, the pH is also lower in blossom honey because honeydew honey contains substances with buffering capacity that affect pH. The natural acidity of honey can be increased by its storage and ripening, as well as during its fermentation (**Živkov-Baloš *et al.*, 2021**).

Increased FA may be related with inappropriate treatment during the honey production, e.g. FA in honey increased during the treatment by formic acid to suppress the varroosis in bee colonies during the summer (**Staroň *et al.*, 2023**).
Mineral substances influence the electrical conductivity. Honey with higher minerals content has higher EC and vice versa. According to the legislation, honeydew honey has EC of 0.8 mS/cm and more, while blossom and blended honeys have EC below this limit, with exceptions of chestnut (*Castanea sativa*), strawberry tree (*Arbutus unedo*), heather (*Erica* spp., *Calluna vulgaris*), eucalyptus, lime tree (*Tilia* spp.), manuka (*Leptospermum scoparium* J. R. et G. Forst.) and tea tree (*Malaleuca* spp.) (**Directive 2014/63/EU, 2014**). Higher electrical conductivity together with higher protocatechuic acid and oligosaccharides content seems to be a good tool for differentiation coniferous honeydew honey from non-honeydew honey (**Recklies *et al.*, 2021**). For the evaluation of honeydew honey, it is advisable to consider the optical rotation of the honey solution, as well as the honeydew elements in its sediment. However, EC is the only parameter given by the applicable legislation in this item.
HMF is formed from monosaccharides or in Maillard reaction, when honey is heated or stored for a long time (**da Silva *et al.*, 2016**). It is a product of acid inversion (**Tomczyk *et al.*, 2023**). Fresh honeys naturally contain small amount of HMF, up to 10 mg/kg. In countries with tropical climate, HMF can be in higher amount. HMF of 28.3 ± 43.9 mg/g was found as overall mean value in honey from Eastern Africa (Ethiopia, Kenya, Sudan, Tanzania, Uganda) (**Mesele, 2021**). HMF content gradually increases under the high temperature acting for a long time, e. g. 70 °C for 720 min (**Ribeiro *et al.*, 2012**). Storage of honey at 4-25 °C seems to be optimal in terms of retaining low levels of HMF for at least 2 years depending on its botanical origin (**Chou *et al.*, 2020**). Proteins and phenolic compounds were identified as important bioactivity indicators (**Tomczyk *et al*., 2023).** These special substances are capable of enhancing the antimicrobial potential of honey (**Tsadila *et al.*, 2023**). Free radicals (mainly reactive oxygen and nitrogen species) are developed *in vivo* during oxidative stress in organisms and they have positive physiological functions (e.g. in anti-inflammatory reactions), but they also act negatively mainly to lipids, proteins and nucleic acids by change of their structure and function (**Paulova *et al.*, 2004**). Mechanism resulting in prevention of oxidation through the pathway of reduction, bleaching or free radical scavenging is highly dependent upon the identity of the antioxidants (**Chua *et al.*, 2015**). In addition to endogenous antioxidants (e.g. glutathione, uric acid, coenzyme Q), honey contains exogenous antioxidants derived from food, primarily vitamins C and E, carotenoids and polyphenolic compounds such as flavonoids, catechins and phenolic acids (**Paulova *et al.*, 2004**). Phenolic compounds are secondary metabolites synthesized by plants as a response to biotic and abiotic stresses (**Santos *et al.*, 2023**). In general, phenolic compounds in the honey represent important plant markers (**da Silva *et al.*, 2016**). Flavonoids originate in nectar, pollen or propolis (**Chou *et al.*, 2020**). From group of phenolic compounds, mainly caffeic acid and its esters, chrysin, galangin, quercetin, acacetin, kaempferol, pinocembrin, pinobanksin and apigenin are predominant in the honey (**Khalil *et al.*, 2010**). In term of phenolic acids, not only caffeic acid, but also gallic, chlorogenic, vanillic, coumaric, rosmarinic, cinnamic and ferulic acid, was confirmed to be present in Lithuanian honey from raspberry (*Rubus ideaus* L.), acacia (*Acacia catechu* Willd.), buckwheat (*Fagopyrum esculentum* Moench), from forest (polyfloral) and in honey from Spanish supermarket originated in eucalyptus (*Eucalyptus globules*) (**Ramanauskiene *et al.*, 2012**). Amount of phenolic acids is influenced by the botanical source of nectar as well as its geographic location (**Almasaudi, 2021**). Along with polyphenols, glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, amino acids and proteins also contribute to antioxidant properties of honey (**Khalil *et al.*, 2010**). Polyphenolic compounds and their interaction with H_2O_2 were found as the key factors responsible for high antibacterial activity of honeydew honey (**Bucekova *et al.*, 2018**). Correlation was found between the colour and content of some biologically active substances (especially phenolics and flavonoids) (**Šubert *et al.*, 2022**). In general, dark honeys are considered to have stronger antioxidant activity compared to light ones. Antioxidant activity was linked with polyphenols, honey colour, and also amino acids and the Maillard reaction products (**Brudzynski, 2012**). Honey contains proteins of plant as well as animal origin (**Machado De-Melo *et al.*, 2018**; **Mureșan *et al.*, 2022**). Their amounts in honey is low in comparison to other substances and depend also on bee species: 0.2-1.6% in *Apis mellifera* honey and 0.1-3.3% in *Apis cerana* honey (**Lee *et al.*, 1998**). Identification of pollen proteins in the honey can serve as floral origin markers (**Balkanska *et al.*, 2020**). Honey is often described as a "living food" due to the presence of enzymes. Enzymes belong to important protein substances in honey. The most known are invertase (β-fructofuranosidase), glucose oxidase (GOX), catalase, diastase (*α*- and *β*-amylase) and acid phosphatase, which come from nectar sources, salivary fluids and gland secretions of honey bee (**Serrano *et al.*, 2007**). Enzymes catalyse chemical reactions. Diastase participates in starch breakdown. Invertase catalyses breakdown of saccharose to glucose and fructose. GOX catalyses the production of gluconic acid and H_2O_2 from glucose in diluted or unripe honey (**White *et al.*, 1963**; **White, 1966**). Next enzyme – catalase – is naturally occurring in some pollen grains and can neutralise H_2O_2, which is broken down into the water and oxygen under the enzyme´s influence (**Weston, 2000**).
Next important group of honey proteins are major royal jelly proteins (MRJPs), also labelled as apalbumins (APAs). They are not only a source of nutrition, but they play a key role in larval development, and participate in defence of honey bees against microbial pathogens. APAs are multifunctional proteins with a broad spectrum of therapeutic properties in human healthcare. Apalbumin1 (APA1), the major protein of royal jelly, is the major and regular protein of honey as well, while the compounds of floral origin in honey can vary significantly. APA1 is honey bee specific and can be considered as the marker of honey authenticity and quality (**Bíliková *et al.*, 2015**; **Bíliková & Šimúth, 2010**; **Chua *et al.*, 2015**). Worker bees inside the hive take the nectar and honeydew from foraging bees and process them further in the hive environment. Before raw material (nectar, honeydew) processing, young bees produce royal jelly and feed the brood and queen. Later, production of royal jelly decreases, and they start to process the feed, and gland secretions are changed. However, they still produce a certain amount of royal jelly proteins, which are detectable in the honey. Honey proteins also have significant antioxidant activity as free radicals' scavengers and reducing agents (**Chua *et al.*, 2015**).
The objective of this study was to characterize the physico-chemical parameters of selected honey samples available in Slovakia. The research aimed to evaluate these parameters across honey samples from various region with a focus on analysing moisture content, pH, electrical conductivity (EC), free acidity (FA), hydroxymethylfurfural (HMF) content, polyphenolic compounds, proteins, amino acids, and their correlation with geographical and botanical origins. The study also aimed to establish correlations between these parameters and assess their potential impact on honey quality.

MATERIAL AND METHODS

Material

Overall, twenty-five *Apis mellifera* honey samples were obtained directly from beekeepers. The samples were divided into three groups based on their botanical origin and into two groups based on their geographical origin. False acacia (*Robinia pseudoacacia*) and rapeseed (*Brassica napus*) honeys were identified by the beekeepers and confirmed by their typical sensory properties, as well as later by their low FA and EC values. Other blossom honeys were evaluated together. Samples collected from apiaries located near forests were labelled as "forest" by beekeepers and evaluated collectively as a third group based on botanical origin. Although this term is not defined in legislation, it is popular among consumers, as this group may include blossom, blended or honeydew honeys. In addition to apiary honey samples, six commercial samples were obtained from shops for comparison with apiary samples. Three of the six commercial samples were purchased from retail outlets and had known beekeepers listed on the labels. The remaining three samples were obtained from chain stores and were labelled as a mixture of EU and non-EU honeys, a common practice in chain retail. In total, thirty-one honey samples were analysed, all of which were available in Slovakia. A more detailed characterisation of the samples is provided in Table 1.

Physico-chemical analyses

Water content, free acidity, pH, electrical conductivity

The parameters were measured according to IHC (**International Honey Commission, 2009**). Water content was found by Abbé refractometer (Carl Zeiss Jena, Germany). Honey acidity (free acidity and pH) was found in honey solution. Honey (10 g) was dissolved in 75 ml distilled water with pH value of 7.0. Values of pH were determined potentiometrically by pH-meter HI 111 (Hanna Instruments, USA). Free acidity was determined by titration of 0.1 mol/dm^3 NaOH to pH of 8.3 and based on the consumption, free acidity was calculated. Electrical conductivity was measured in 20% w/v honey solution (20 % of honey dry matter) by conductometer CO 3100 L (VWR®, Germany).

Hydroxymethylfurfural

The 5-hydroxymethylfurfural (HMF) content in honey was determined using HPLC-UV according to the IHC (**International Honey Commission, 2009**) with modifications in the chromatographic conditions (**Kukurová *et al.*, 2023**). A HPLC Series 1200 (Agilent Technologies, USA) with Zorbax C18 SB column (250 × 4.6 mm, 5 μm) tempered to 30 °C and mobile phase consisted of water (A), methanol (B) and acetonitrile (C) with a gradient elution at a flow rate of 0.8 ml/min were used. An amount of 1 g of sample was dissolved in 10 ml of methanol: deionized water (80:20, v/v) extract solution using an ultrasonic bath for 5 min, a vortex, and a rotatory shaker for 30 min. After centrifugation and filtration through a nylon syringe filter (0.45 μm), samples were transferred to 2 ml glass vials to injection. The retention time of HMF was 10.8 min and the total time of analysis was 37 min. The HMF content in honey samples was calculated using the calibration curve prepared from a stock HMF solution (1 g/l) in a linear range from 1 mg/l to 100 mg/l. Results were expressed in mg/kg with LOD = 0.5 mg/kg and LOQ = 1.7 mg/kg.

Table 1 Characterization of the analysed samples

Sample code	Botanical origin	Geographical origin	Source (obtaining)	Production/ Packaging/ BB (best before)
FaSBee1	Blossom (false acacia)	Lowlands (Veľký Krtíš)		
RaSBee1	Blossom (rapeseed)	Lowlands (Trebišov)		
FaSBee2	Blossom (dominance of false acacia)	Lowlands (Veľký Krtíš)		
RaSBee2	Blossom (dominance of rapeseed)	Lowlands (Trebišov)		
FaSBee3	Blossom (false acacia)	Lowlands (Trebišov)		
FaSBee4	Blossom (dominance of false acacia)	Lowlands (Sobrance)		
FoSBee1	Forest* + sunflower	Lowlands (Porúbka – Sobrance)		
BloSBee1	Blossom (false acacia + linden)	Highlands (Kšinná – Rakovec)		
BloSBee2	Blossom	Highlands (Smrečany)		
BloSBee3	Blossom (meadow flowers)	Highlands (Dolný Pajer)		
FoSBee2	Forest*	Highlands (Pavčina Lehota)		
BloSBee4	Blossom (creamed)	Highlands (Liptovský Mikuláš)		
BloSBee5	Blossom	Highlands (Rakovec)	Apiary	2021
BloSBee6	Blossom (hawthorn + fruit trees)	Highlands (Dolný Pajer)		
BloSBee7	Blossom	Highlands (Gerlachov)		
FoSBee3	Forest*	Highlands (Jánska dolina - L. Ján)		
FoSBee4	Forest*	Highlands (Reľov)		
BloSBee8	Blossom (sunflower)	Lowlands (Trebišov)		
BloSBee9	Blossom (linden)	Highlands (Dolný Pajer)		
FoSBee5	Forest*	Highlands (L. Lužná)		
FoSBee6	Forest*	Highlands (L. Hrádok – Fabriky)		
FoSBee7	Forest*	Highlands (Bocka dolina)		
FoSBee8	Forest* (forest meadows)	Highlands (Levočské vrchy)		
FoSBee9	Forest*	Highlands (L. Hrádok – Maša)		
FoSBee10	Forest*	Highlands (L. Hrádok)		
BloSCo1	Blossom	Lowlands (Rudno nad Hronom)		
FoSCo1	Forest*	Lowlands (Rudno nad Hronom)	Retail	packed 2021
BloSCo2	Blossom (raspberry)	Highlands (L. Hrádok)		
FoFCo1	Forest (blended)	EU and non-EU		BB 2024
BloFCo1	Blossom	EU and non-EU	Chain store	BB 2024
BloFCo2	Blossom	EU and non-EU		BB 2022

sample code: Fa - false acacia, Ra - rapeseed Blo = blossom, Fo - forest (*marked by beekeeper); S = Slovak, F = foreign (mixture of EU and non-EU honeys); Bee = directly from beekeeper, Co = commercial (from the trade) + number; false acacia – *Robinia pseudoacacia*, rapeseed – *Brassica napus*, sunflower – *Helianthus annuus*, linden – *Tilia* spp., hawthorn – *Crataegus laevigata*, raspberry – *Rubus ideaus*, EU – European Union, highlands - apiary altitude more than 300 meters above sea level, lowlands – apiary altitude up to 300 meters above sea level

Sample preparation before determination of AA (by DPPH• method) and TPC spectrophotometric assay

An amount of 1 g of sample was extracted with 20 ml of 80 °C distilled water for 10 minutes. After centrifugation at 4000 *g* (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, *total phenolic content*). Extraction was carried out in triplicate. All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Radical scavenging activity – DPPH• method

Radical scavenging activity of extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (**Sánchez-Moreno *et al.*, 1998**). The sample (0.4 ml) was mixed with 3.6 ml of DPPH• solution (0.025 g DPPH• in 100 ml methanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-100 mg/l; R^2=0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Total phenolic content (TPC)

The extracts for TPC evaluation were measured by the method of **Singleton & Rossi** (**1965**) using Folin-Ciocalteu reagent and 0.1 ml of each sample was mixed with 0.1 ml of the Folin-Ciocalteu reagent, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25-300 mg/l; R^2=0.998) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

Determination of protein concentration

The protein content was determined using the microplate assay, according to **Bradford** method **(1976)**. To the 100 μl of the sample or its dilution in physiological solution, was added 100 μl of Quick Start Bradford reagent (BioRad, Laboratories, Inc., USA). The absorbance was measured at 595 nm. Bovine serum albumin (BSA, Sigma, USA) was used as a standard. Each sample was analysed at three dilutions and each dilution in three parallel analyses.

Determination of Apalbumin 1 and RJPs in honeys by ELISA

Honey samples were analysed for APA1 quantification as described previously in detail (**Bíliková & Šimúth, 2010**). The 96 well/flat-bottom microtiter plates (Brand, Germany) were coated of antigen - diluted honey samples at dilution of 0.05% and/or 0.001% in milliQ water and/or standard solution of APA1 and incubated overnight at 4 °C. After washing with TBS buffer (100 mmol/L Tris and 150 mmol/L NaCl, pH 7.5) the plates were incubated with polyclonal rabbit anti-apa1 antibody or rabbit anti-RJPs antibody respectively, in milk buffer (2% non-fat milk in TBS) and then, with peroxidase-conjugated anti-rabbit antibody in milk buffer for 1 h. Detection was done by adding of 3% ABTS (2,2´-azino-bis-(3-benzthiazoline-6-sulfonic acid, Southern Biotech, USA), in 50 mmol/L citrate buffer pH 4.3; supplemented by hydrogen peroxide. The absorbance at 405 nm was read in a Microplate Spectrophotometer PowerWaveTM XS (BioTek Instruments, INC, Winooski, Vermont, USA). Data processing and statistic were performed with the Gen5 software (BioTek, USA).

Statistical analysis

Program Jamovi version 2.3.18.0 (***jamovi - open statistical software for the desktop and cloud*, n.d.**) was used for descriptive statistics and for comparison of values from evaluated honey groups. Differences between the variables (i.e. significance of the parameter as factor) were evaluated by non-parametric Kruskal-Wallis's test and between the honey groups in individual parameters by Dwass-Steel-Critchlow-Fligner pairwise comparisons.

Multiple regression analysis was used for explanation of relationships between electrical conductivity and other explanatory variables. It was realized by program R of version 4.3.0. Firstly (before multiple regression) data were z-scaled because values of variables had different scale. Explanatory variables, which have no significant impact on explanation of relationship, were gradually excluded from linear model based on marginality rules.

Packages „ggplot2" (**Wickham, 2011**) a „GGally" (**Schloerke *et al.*, 2018**) of program R were used for graphical view of data.

RESULTS

Physico-chemical parameters in apiary samples of honey

Honeys divided according to botanical origin

Physico-chemical parameters of the honeys analysed according their botanical origin are shown in Table 2.

Water content was found in accordance with the maximum limit of 20% established by European legislation (**Council Directive 2001/110/EC, 2001**). The differences between evaluated honeys were not statistically significant. The risk of fermentation decreases with lower water content. Water content is an important parameter for the long-term storage of honey to maintain its quality without any defects.

Table 2 Physico-chemical parameters in apiary samples divided according to botanical origin

Parameter	Botanical origin	n	Mean ± SD	Min	Max
Water (%)	Blossom (false acacia, rapeseed)	6	17.8 ± 0.3	17.3	18.2
	Blossom	9	17.9 ± 1.4	16.2	19.8
	Forest	10	17.4 ± 1.6	14.8	20.0
pH	Blossom (false acacia, rapeseed)	6	3.90 ± 0.11	3.80	4.11
	Blossom	9	3.99 ± 0.13	3.81	4.20
	Forest	10	4.29 ± 0.42	3.82	4.98
FA (meq/kg)	Blossom (false acacia, rapeseed)	6	$14.5 \pm 1.8^{a*}$	12.0	17.0
	Blossom	9	$26.4 \pm 6.3^{b*}$	19.5	35.8
	Forest	10	$24.3 \pm 10.5^{ab*}$	11.0	41.5
EC (mS/cm)	Blossom (false acacia, rapeseed)	6	$0.174 \pm 0.031^{a*}$	0.141	0.228
	Blossom	9	$0.403 \pm 0.072^{bc*}$	0.315	0.530
	Forest	10	$0.557 \pm 0.171^{cb*}$	0.292	0.805
HMF (mg/kg)	Blossom (false acacia, rapeseed)	6	5.06 ± 1.38	3.58	7.26
	Blossom	9	6.01 ± 1.99	3.65	9.59
	Forest	9	7.26 ± 10.96	1.62	36.22
AA (mg TEAC/g)	Blossom (false acacia, rapeseed)	6	0.15 ± 0.01^{a}	0.13	0.16
	Blossom	9	0.26 ± 0.07^{bc}	0.18	0.39
	Forest	10	0.26 ± 0.05^{cb}	0.20	0.39
TPC (mg GAE/g)	Blossom (false acacia, rapeseed)	6	0.28 ± 0.15^{a}	0.14	0.55
	Blossom	9	0.43 ± 0.14^{ab}	0.14	0.65
	Forest	10	0.71 ± 0.73^{b}	0.34	2.75
Proteins (μg/ml)	Blossom (false acacia, rapeseed)	6	827.69 ± 405.21	552.50	1631.46
	Blossom	9	1079.58 ± 221.90	807.54	1498.02
	Forest	10	1253.45 ± 291.45	895.74	1772.18
RJPs (μg/ml)	Blossom (false acacia, rapeseed)	6	500.86 ± 218.99^{ac}	358.96	938.20
	Blossom	9	876.81 ± 133.53^{b}	626.48	1001.00
	Forest	10	677.06 ± 172.81^{ca}	468.13	1008.76
APA 1 (μg/ml)	Blossom (false acacia, rapeseed)	6	251.63 ± 65.39	161.07	357.14
	Blossom	9	414.85 ± 135.62	232.38	627.20
	Forest	10	378.91 ± 121.92	190.07	554.40

Triplets for each parameter in column were statistically analysed. Different letters in column indicated significant differences ($p < 0.05$, $*p < 0.01$).

Blossom honey from rapeseed and false acacia had significantly lower ($p < 0.01$) free acidity and electrical conductivity compared to other honeys.

HMF, an indicator of honey freshness and appropriate processing without exposure to high temperatures, was found to be below 10 mg/kg in all evaluated samples. No significant differences were observed between the evaluated groups. The maximum allowable limit for HMF is 40 mg/kg in general and 80 mg/kg for honey with declared origin in tropical countries (**Council Directive 2001/110/EC, 2001**). In one case, the HMF value reached 36.22 mg/kg, which is in accordance with legislation; however, this value is considered high for fresh honey. The HMF content for forest honey was calculated from nine samples, as one sample (FoSBee9) had HMF levels below the limit of quantification (LOQ).

False acacia and rapeseed honey had lower antioxidant activity compared to other blossom and forest honeys and lower total phenolic content compared to forest honey. The total phenolic content values were mostly below 0.82 mg GAE/kg. However, in one case, a high TPC value of 2.75 mg GAE/kg was found in a forest honey sample (FoSBee6).

The soluble protein fraction analysis showed no significant differences. However, significant differences in RJPs content were found. The highest mean RJPs value was found in blossom honey and was significantly higher compared to false acacia, rapeseed, and forest honey.

Honeys divided according to geographical origin

Results of apiary honeys divided according to altitude of the apiary are shown in Table 3. Honeys from highlands had significantly higher pH, FA, EC and RJPs content. The differences were probably caused by a higher number of samples from highlands as well as more diverse pasture in highlands, in general. HMF below LOQ was detected in one sample from highlands (FoSBee9).

Table 3 Physico-chemical parameters in apiary samples divided according to geographical origin

Parameter	Geographical origin	n	Mean ± SD	Min	Max
Water (%)	Highlands	17	17.8 ± 1.4	16.2	20.0
	Lowlands	8	18.0 ± 1.1	14.8	18.2
pH	Highlands	17	4.17 ± 0.35^{a}	3.81	4.98
	Lowlands	8	3.91 ± 0.10^{b}	3.80	4.11
FA (meq/kg)	Highlands	17	25.0 ± 8.6^{a}	11.0	41.5
	Lowlands	8	17.9 ± 7.7^{b}	12.0	35.8
EC (mS/cm)	Highlands	17	$0.494 \pm 0.154^{a**}$	0.315	0.805
	Lowlands	8	$0.232 \pm 0.125^{b**}$	0.141	0.515
HMF (mg/kg)	Highlands	16	4.66 ± 1.98	1.62	9.59
	Lowlands	8	9.40 ± 10.97	3.58	36.22
AA (mg TEAC/g)	Highlands	17	0.24 ± 0.04^{a}	0.18	0.35
	Lowlands	8	0.21 ± 0.11^{b}	0.13	0.39
TPC (mg GAE/g)	Highlands	17	0.56 ± 0.57	0.14	2.75
	Lowlands	8	0.40 ± 0.25	0.14	0.81
Proteins (μg/ml)	Highlands	17	1145.18 ± 270.46	807.54	1772.18
	Lowlands	8	968.60 ± 434.29	552.50	1631.46
RJPs (μg/ml)	Highlands	17	779.70 ± 173.07^{a}	506.44	1008.76
	Lowlands	8	551.51 ± 242.64^{b}	358.96	938.78
APA1 (μg/ml)	Highlands	17	392.16 ± 129.32	190.07	627.20
	Lowlands	8	295.72 ± 110.31	161.07	520.59

Duplets for each parameter in column were statistically analysed. Different letters in column indicated significant differences ($p < 0.05$, $**p < 0.001$).

Correlations between the parameters evaluated

Correlation was found between the EC, FA, and pH in Slovak honey obtained directly from beekeepers. A graphical view of the relationship is shown in Figure 1.

EC is a closely related to FA and pH. These two parameters participate on prediction of EC correct value by 84.27% (adjusted $R^2 = 0.8427$). Prediction of this relation was expressed as EC = -1.961952 + 0.496727×pH + 0.015075×FA. EC value predicted by this way will be correct mainly in false acacia and rapeseed Slovak honey as well as honey from Slovak forests, because pH and FA showed moderate correlation with EC in these honey types.

Rapeseed and false acacia honey are characterized by low FA and EC. Honeys from forest apiaries, whether blossom polyfloral or blended or honeydew, have higher FA and EC.

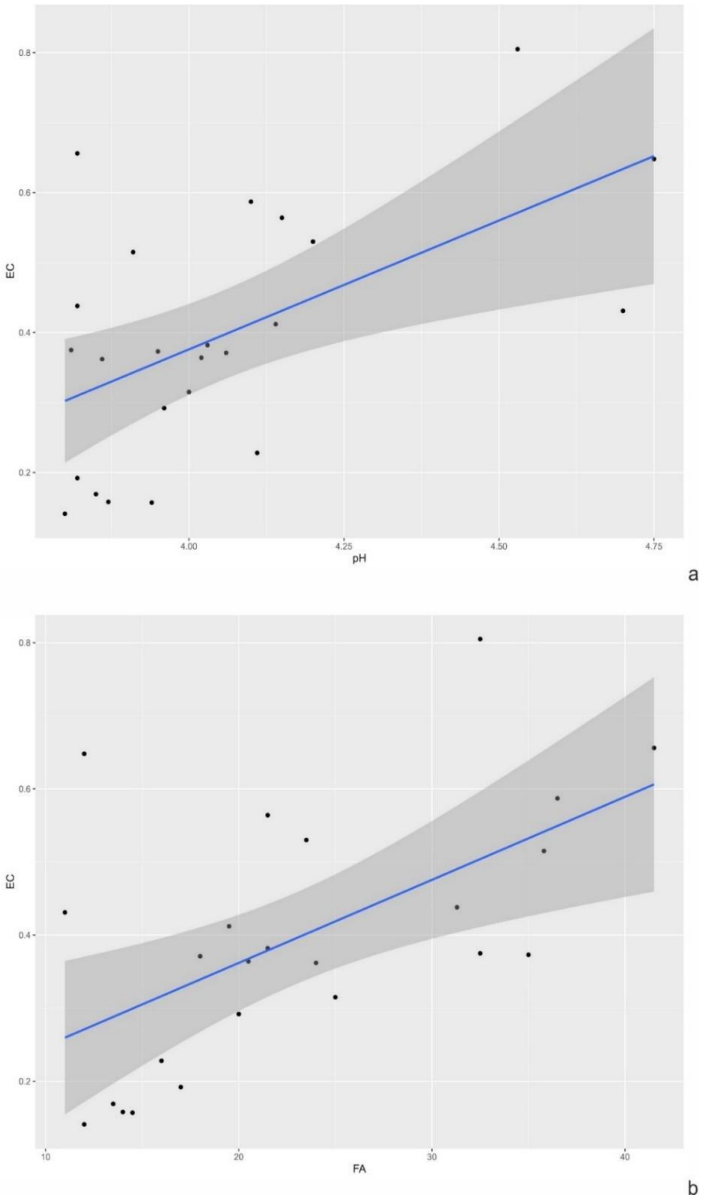

Figure 1 Relationship between electrical conductivity and acidity (represented by pH – part a, and free acidity – part b) in the honey from Slovak apiaries

Figure 2 shows the correlation between electrical conductivity (EC), pH, and free acidity (FA) in Slovak honey samples, divided by botanical origin (part a) and geographical origin (part b).

In part a, correlations vary substantially between honey types, where Blossom honeys (red) show a strong negative correlation between pH and FA (r = –0.613), and moderate correlations between EC and pH (r = 0.381) as well as EC and FA (r = 0.204). False acacia + rapeseed honeys (green) exhibit a strong positive correlation between EC and FA (r = 0.791) and between EC and pH (r = 0.740), indicating that both acidity and mineral content are more consistently linked in these more uniform floral sources. Forest honeys (blue) show a strong negative correlation between pH and FA (r = –0.608), but weaker correlations involving EC.

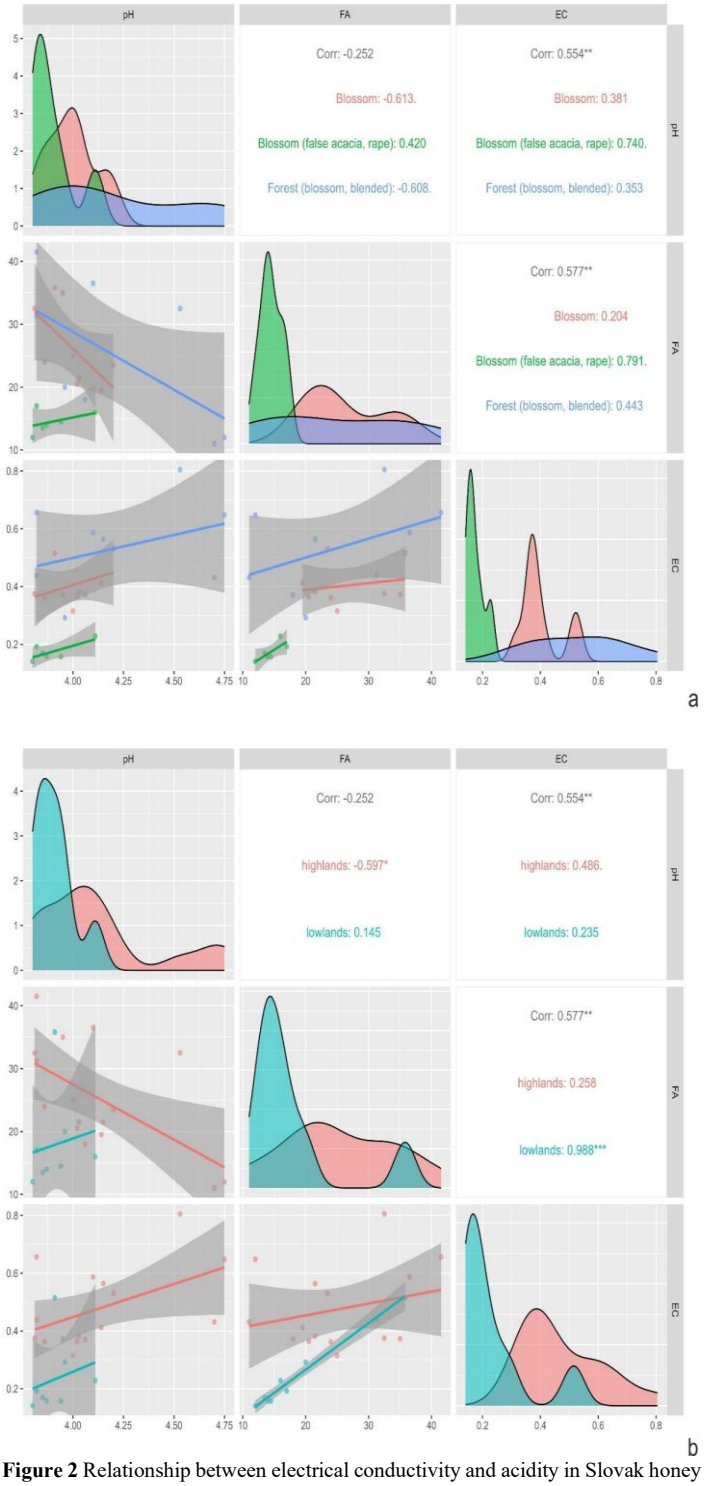

Figure 2 Relationship between electrical conductivity and acidity in Slovak honey divided by its botanical (part a) and geographical origin (part b)

correlations: + positive, - negative, *moderate, **moderate to strong, ***strong; The first number in the square - Corr. is correlation coefficient between individual parameters (pH, FA, EC) for all apiary samples and next numbers are correlation coefficients for evaluated groups (false acacia and rapeseed, blossom, forest, from highlands and lowlands)

In part b, where honey samples were grouped by altitude, the Highland honeys (pink) exhibit a moderate negative correlation between pH and FA (r = –0.597*) and a weak-to-moderate correlation between EC and both acidity parameters. Lowland honeys (cyan) show a very strong positive correlation between EC and FA (r = 0.988***), indicating a tight relationship between acidity and conductivity in this group, possibly due to lower environmental variability or more homogeneous botanical sources.

Overall, the figure demonstrates that the relationship between EC and acidity depends strongly on both botanical and geographical origin, with the strongest association observed between free acidity and EC in lowland honeys.

Physico-chemical parameters in honey samples according to their source of obtaining

There were no significant differences in water content, pH, FA and EC among honeys obtained from different sources (apiary, retail, chain store). However, HMF levels in apiary samples were significantly lower than in commercial samples (Table 4). HMF below LOQ was detected in one apiary sample (FoSBee9). Higher HMF values could indicate older or heated honey. Additionally, significantly higher levels of proteins, including RJPs were found in apiary samples compared to commercial samples (Table 4).

Table 4 Physico-chemical analysis of tested honey divided according to the source of its obtaining

Parameter	Source	n	Mean ± SD	Min	Max
Water (%)	Apiary	25	17.7 ± 1.3	14.8	20.0
	Retail	3	17.6 ± 0.4	17.2	18.0
	Chain store	3	17.3 ± 0.6	16.7	17.9
pH	Apiary	25	4.09 ± 0.32	3.80	4.98
	Retail	3	3.96 ± 0.26	3.81	4.26
	Chain store	3	4.24 ± 0.08	4.17	4.33
FA (meq/kg)	Apiary	25	22.7 ± 8.8	11.0	41.5
	Retail	3	15.0 ± 5.6	9.0	20.0
	Chain store	3	15.9 ± 4.6	12.3	21.0
EC (mS/cm)	Apiary	25	0.410 ± 0.189	0.141	0.805
	Retail	3	0.251 ± 0.090	0.147	0.312
	Chain store	3	0.277 ± 0.130	0.133	0.387
HMF (mg/kg)	Apiary	24	6.24 ± 6.66^{a}	1.62	36.22
	Retail	3	73.08 ± 54.32^{bc}	10.79	110.60
	Chain store	3	23.85 ± 8.03^{cb}	17.26	32.79
AA (mg TEAC/g)	Apiary	25	0.23 ± 0.07	0.13	0.39
	Retail	3	0.22 ± 0.04	0.18	0.26
	Chain store	3	0.20 ± 0.05	0.15	0.25
TPC (mg GAE/g)	Apiary	25	0.51 ± 0.49	0.14	2.75
	Retail	3	0.30 ± 0.09	0.24	0.40
	Chain store	3	0.46 ± 0.08	0.40	0.55
Proteins (μg/ml)	Apiary	25	1088.67 ± 332.94^{a}	552.50	1772.18
	Retail	3	713.32 ± 63.55^{ab}	672.50	786.54
	Chain store	3	506.89 ± 258.91^{b}	213.08	701.66
RJPs (μg/ml)	Apiary	25	706.68 ± 221.23^{a}	358.96	1008.76
	Retail	3	306.30 ± 74.91^{bc}	219.84	351.72
	Chain store	3	210.78 ± 133.95^{cb}	68.10	333.84
APA 1 (μg/ml)	Apiary	25	361.30 ± 129.64	161.07	627.20
	Retail	3	188.25 ± 82.48	119.15	279.56
	Chain store	3	200.17 ± 122.93	61.37	295.31

Triplets for each parameter in column were statistically analysed. Different letters in column indicated significant differences ($p < 0.05$).

DISCUSSION

The commercial honey samples included in this study were selected to represent the diversity of products available on the Slovak market, which includes both EU and non-EU origin honeys. While this selection provides insight into the range of honey products available to consumers, it may also introduce variability due to differences in geographical origin, botanical sources, and processing methods. This should be considered when interpreting the results, as the primary focus of this study remains the characterization of Slovak apiary honeys.

Physico-chemical quality of apiary honey samples

Apiary honey samples according to botanical origin

No significant differences in water content were found among the analysed honeys. Similar results were reported for Serbian honey, where water content ranged from 14.00 ± 0.48% to 19.22 ± 0.65%, while higher values of water content were observed in blossom honey compared to honeydew honey (**Matović *et al.*, 2018**). Honeys with higher water content are more sensitive to fermentation. Honey spoilage can be investigated by yeast count and the determination of glycerol, butanediol or ethanol (**Ruoff & Bogdanov, 2004**).

The pH values did not show significant differences. Generally, honey pH ranges between 3.2 and 4.5 (**Kwakman & Zaat, 2012**). Blossom honeys typically exhibit lower pH, whereas honeydew honeys can reach higher values, approximately up to 5.7 (**Oddo *et al.*, 2004**). For example, pH values ranged from 3.95 to 4.75 in Polish blossom honey (**Vîjan *et al.*, 2023**). In the honey from Austria and Slovakia, the highest FA was determined in chestnut and honeydew honey while rapeseed and false acacia honey showed the lowest FA and pH (**Kukurová *et al.*, 2023**). In general, false acacia and rapeseed honeys have lower FA and EC compared to other honeys (**Oddo *et al.*, 2004**), which is confirmed by our results as well as by the results from Romanian honey (**Vîjan *et al.*, 2023**), particularly regarding EC."

In Serbian honeys labelled as forest, a wide range of EC values was found – from 0.09 to 1.99 mS/cm – indicating a diverse botanical origin of the evaluated samples (**Živkov Baloš *et al*., 2018**). A very high EC value, specifically 4.18 ± 0.05 mS/cm, was reported for Yemeni honey (**El Sohaimy *et al*., 2015**). In European honeys, the highest EC values were found in honeydew honeys (ranging from 0.85 to 1.63 mS/cm), and in special Metcalfa honeydew honeys (ranging from 1.21 to 2.17 mS/cm) (**Oddo *et al*., 2004**).Determining the blended origin of honey is difficult, as it is not supported by specific parameter limits in the current legislation. The label "forest honey" is commonly used in various countries. According to recent research, all Austrian honeys labelled as "forest" had EC values above 0.8 mS/cm, indicating their honeydew origin (**Kukurová *et al.*, 2023**). In the case of honeys labelled as "forest" in our study, the botanical origin was likely floral, mixed, or honeydew-based, as indicated by the variation in electrical conductivity. Although the term "forest honey" is widely used by beekeepers and consumers, it often evokes honeydew honey, which may not always reflect the actual composition.

The content of HMF in fresh, unprocessed honey is often below the detectable limit or very low, while higher HMF shortly after extraction can range from 10 to 15 mg/kg (**Thrasyvoulou *et al.*, 2018**). In Ukrainian honey, HMF levels ranged from 1.6 ± 0.1 to 7.9 ± 0.2 mg/kg (**Adamchuk *et al.*, 2020**). Taiwanese honey contained HMF levels between 1.21 and 2.13 mg/kg after extraction, which increased to the maximum limit for tropical honey (80 mg/kg) and more after storage at 35 °C for 6 - 9 months (**Chou *et al.*, 2020**). Low HMF content was observed in most apiary samples, except for one honey that had probably undergone heat processing. However, similar results were published about Andalusian honey (range from 0.19 to 41.16 mg/kg with mean value of 8.24 mg/kg), where one sample contained slightly higher HMF as general limit (40 mg/kg) and authors (**Serrano *et al.*, 2007**) took into consideration the climatic conditions of Andalusia, which could increase the formation of HMF. The Slovak sample BloSBee7 contained 36.22 mg/kg HMF and was sourced from Porúbka-Sobrance (lowland – 172 m), where warm weather conditions are possible. In the time of climatic change all over the world, when average temperatures are increasing, it is necessary to analyse honeys in terms of HMF because of possible natural HMF increase in some types of honey.

In Polish honey, high TPC (total phenolic content) was found in buckwheat, honeydew and heather honey while manuka honey from New Zealand was used as positive control with the highest TPC (**Pentoś *et al.*, 2020**). In Romanian honey, lower TPC was observed in rapeseed, false acacia and also linden honey (**Vîjan *et al.*, 2023**), what is partially in accordance with our results – lower antioxidant activity in false acacia and rapeseed honey compared to other honeys and lower number of TPC compared to forest honey. However, rapeseed and buckwheat honey from Moldova had higher amount of TPC as lavender honey from the same country (**Chirsanova *et al.*, 2021**). The highest TPC was found in honeydew honey compared to blossom honeys, however all parameters related to antioxidant activity were not the highest in honeydew honey in the experiment (**Meda *et al.*, 2005**). A comparable range of TPC from 26 to 159 mg GAE/100 g (i.e. 0.26 – 1.59 mg GAE/g) was reported in honey predominantly composed of Fabaceae pollen with minor contributions from Asteraceae, Tiliaceae, Myrtaceae and Apiaceae (**Otmani *et al.*, 2022**). Similarly, honey from Burkina Faso exhibited TPC ranging

from 32.59 to 114.75 mg GAE/100 g (**Meda *et al.*, 2005**). The variation of TPC among individual plants is primarily influenced by soil composition, growing environment, cultivation techniques and genetics (**Behl *et al.*, 2021**).
Special jujube (*Ziziphus lotus* L.) honey from Algeria was tested, revealing protein content of 577 ± 180 μg/g (from 484 to 667 μg/g), which was considered as moderate (**Zerrouk *et al.*, 2018**). In comparison, Slovak samples exhibited higher protein content values. Honeys from New Caledonia, known for their medical grade potential, were analysed and found to have a similar range of protein content (448 - 1282 μg/g) (**Bucekova *et al.*, 2023a**).
Rapeseed and false acacia honey showed lower antioxidant activity and RJPs content. Rapeseed and false acacia honey had naturally lower FA and EC (**Oddo *et al.*, 2004**). Lower AA and RJPs content seem to be also a natural property. On the other hand, *Brassica* plants, including vegetables like cabbage or broccoli, contain compounds with pharmacological activity, known to prevent chronic diseases and cancer (**Jan *et al.*, 2018**; **Mandrich & Caputo, 2020**). Brassinosteroids are plant hormones necessary to normal growth and development of plant (**Clouse, 2011**). They provide resistance against viruses and also act as anticancer agent (**Tileuberdi *et al.*, 2022**). Nectar is rich in phytohormones, including brassinosteroids, and its presence was confirmed also in the honey (**Wang *et al.*, 2017**). In summary, each type of honey possesses unique properties and potential applications derived from these properties.

Apiary honey samples according to geographical origin

The physico-chemical properties of honey are influenced by multiple factors, including bee species, floral sources, and environmental conditions. Legislative regulations primarily apply to *Apis mellifera* honey (**Codex Standard for Honey, 2022**; **Council Directive 2001/110/EC, 2001**; **Directive 2014/63/EU, 2014**), whereas honey from stingless bees (*Melipona* spp.) typically exhibits higher water content and acidity (**Moo-Huchin *et al*., 2015**; **Ramírez-Miranda *et al.*, 2021**).
In this study, honey samples from different Slovak regions exhibited variations in water content, free acidity (FA), electrical conductivity (EC), and hydroxymethylfurfural (HMF) content, reflecting differences in floral composition and environmental conditions. Samples from lowland areas tended to have higher moisture content, likely due to differences in climate and humidity, while those from higher altitudes exhibited slightly higher acidity, which aligns with previous findings on the influence of plant-derived organic acids (**Oddo *et al*., 2004**).
Previous research has demonstrated that altitude significantly affects honey composition. **Suleiman *et al.* (2020)** found that altitude influences the total phenolic content (TPC) and total flavonoid content (TFC), with effects varying based on floral origin. Similarly, **Grassi *et al.* (2025)** reported that the total phenolic and flavonoid contents, along with the multimineral profile of multifloral honeys, are shaped by altitude and agro-climatic conditions, which are characterized by distinct soil properties and floral biodiversity. Their study found a negative correlation between altitude and total phenolic content but a positive correlation between altitude and total flavonoid content.
Further supporting this, **Scholtz *et al.* (2020)** analysed 112 honey samples, evaluating parameters such as moisture, acidity, pH, hydroxymethylfurfural (HMF), diastase activity (DA), sugars, proline (Pro), electrical conductivity (EC), colour absorbance at 635 nm (C635), and CIELAB colour parameters. Their findings highlight the utility of multivariate analysis for studying the geographical indication of honey.
A comparison with previously published data confirms that Slovak honeys share similarities with European honeys in terms of moisture and acidity levels, aligning with findings from **da Silva *et al.* (2016)**. However, differences were noted when comparing with honeys from Asia and Africa, where lower water content and higher HMF values have been reported (**da Silva *et al*., 2016**).
The geographical location of an apiary plays a critical role in honey composition, particularly when considering rural versus urban environments and pollution levels. Some studies suggest that urban honey may contain higher levels of pollutants but still retain strong antioxidant properties (**Chan *et al*., 2024**). The results of this study support findings from Poland (**Nicewicz *et al*., 2021**), where honey from rural areas exhibited significantly higher antioxidant activity. This suggests that, despite potential contamination from agricultural practices, rural honey may preserve valuable bioactive compounds.
Overall, these findings confirm that geographical origin significantly influences honey composition, reinforcing prior literature while also providing novel insights into Slovak honeys, which remain underexplored. Future research should further investigate the impact of urbanization and environmental pollution on honey quality in Slovakia.

Correlations between the parameters evaluated

Correlations between EC, FA, and pH were observed mainly in false acacia, rapeseed, and forest honey. Weak correlation between pH and FA was found in Slovak and Austrian honey (**Kukurová *et al.*, 2023**).
No correlations were found between AA and TPC in the results. Conversely, in Algerian honey, very high correlations were observed between colour, antioxidants and antioxidant activities (**Otmani *et al.*, 2022**).

Physico-chemical quality of honey according to source of its obtaining

Significant differences were observed in HMF content among the evaluated honey groups. The lowest HMF levels were found in apiary honeys, while the highest mean value was observed in honey obtained from retail. This high mean value was primarily due to a single sample (BloSCo2) with elevated HMF content. After discussing with the producer, it was revealed that the cause was a malfunction of the honey heater resulting in the honey being exposed to a temperature higher than intended (approximately 70 °C instead of the planned 50 °C) during the liquefaction process. Most consumers prefer liquid honey, but higher temperatures can lead to an increase in HMF content and decrease in enzyme activity. This issue may represent a common mistake among small producers. In contrast, large companies, that produce honey for chain stores likely implement standardized processing practices, which helps ensure compliance with the key parameters set by legislation. However, it is essential to analyse these products in more detail to obtain comprehensive information.
Significantly higher protein content was found in apiary honeys compared to honeys from the chain store, which is in accordance with other published studies. In other Slovak samples, protein content was comparable between honeydew honeys (674.6 ± 139.1 μg/g) and blossom honeys (657.7 ± 286.4 μg/g) while samples from supermarkets showed lower protein content: 307.5 ± 153.2 μg/g (**Bucekova *et al.*, 2023b**). Similar results were found in honey from Belgium, France, Italy, Romania and Spain, where protein content ranged from 0.16 to 2.15 mg/ml (**Mureşan *et al.*, 2022**).
As shown in Table 2, the content of water-soluble proteins in the analysed honey samples varied from 827.69 ± 405.21 μg/ml (false acacia, rapeseed honey), 1079.58 ± 221.90 μg/ml (blossom honey) to 1253.45 ± 291.45 μg/ml (forest honey). The major proportion of these proteins consisted of RJPs, accounting for 54,02% to 81,21% of the total protein content in forest and blossom honey, respectively.
The highest dispersion of the values of total water-soluble proteins was observed in false acacia and rapeseed honey (SD ±405.21 μg/ml, that means 48.99% of the average value), as well as in case of RJPs (SD ±218.99 μg/ml, 43.72% of the average value), while in the blossom and forest honey the SD value represented from 15,23% to 25,5% of the average of total protein content and/or the RJPs respectively. Apalbumin1, as a stable parameter of the honey, represented 33% of total protein content on average and 51% of RJPs content respectively with dispersion of 30% of the average values (Table 2).
Besides the proteins of botanical origin, RJPs represent a significant part of proteins in honey. The major royal jelly protein - apalbumin1 (APA1) is the major protein of honey as well and is always present in all honey samples. The amount of the apalbumin1 in honey depends on the properties of the nectar from the given plants. Specifically, nectar with low viscosity (e.g. false acacia nectar) is easier for honey bees to process into honey compared to more viscous nectar (e.g. chestnut nectar). As a result, the content of APA1 is lower in false acacia honey compared to chestnut honey. Blossom and forest honeys are produced by honey bees from the nectar of various plants, leading to greater variability in APA1 content compared to monofloral honeys, which means, the distribution of the APA1 values (SD) can be broader. Concerning the adulterated honeys, the content of APA1 drops down depending on the degree of adulteration of the honey (**Bíliková & Šimúth, 2010**).
The results indicated that the source of honey acquisition is crucial for its quality. Honeys from supermarkets may exhibit changes in biological activity, including antibacterial properties, due to mishandling and improper storage (**Deglovic *et al.*, 2023**).

CONCLUSION

Differences were found in evaluated parameters among honey samples of different botanical origins. Honeys with low electrical conductivity and free acidity (from *Brassica napus* and *Robinia pseudoacacia*) showed significantly lower antioxidant activity, as well as lower content of phenolic compounds (TPC) and RJPs compared to other honey groups. Geographical localization (highland vs. lowlands) also influenced these parameters, because the bee pasture in highlands offering more diverse flora in general. More diverse pasture can result in honeys with a broader spectrum of biologically active substances. Differences were also observed in terms of honey acquisition. Basic analysis showed significantly lower level of hydroxymethylfurfural in apiary samples compared to honeys from retail and chain stores, indicating generally fresher apiary samples. Special physico-chemical analysis revealed significantly higher values of proteins, including RJPs in apiary samples compared to commercial ones. This study demonstrated the high quality of honey from Slovak apiaries, while indicating potential quality deterioration in honey from chain stores, particularly in terms of HMF and proteins, including RJPs. The significant differences observed in protein content and antioxidant properties highlight the potential of these parameters as biomarkers for honey quality and geographical origin. Such indicators could contribute to improved traceability, product labelling, and promotion of Slovak honey as a valuable functional food with health-promoting properties.

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