

## THE EFFECT OF STORAGE TIME ON THE HONEY QUALITY

Jana Tkáčová, Juraj Čuboň, Adriana Pavelková, Marek Bobko, Peter Haščík

### Address(es):

Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, 949 76, Nitra, Tr. A. Hlinku 2, Slovakia.

\*Corresponding author: [jana.tkacova@uniag.sk](mailto:jana.tkacova@uniag.sk)

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### ABSTRACT

The aim of the study was to evaluate the quality of foreign honeys purchased in the Slovak trade network during storage at temperatures of 4 and 20 °C. In the study, we monitored pH, electrical conductivity, colour intensity, water content, total soluble solids, free acidity, and hydroxymethylfurfural (HMF) content. All honeys met the legislative requirements of the Slovak Republic and the European Union, except for the electrical conductivity of avocado honey (1.46 to 1.67 mS.cm<sup>-1</sup>), while Slovak and European legislation sets a limit of 0.8 mS.cm<sup>-1</sup>. The content of HMF in false acacia honey from Hungary reached the threshold value of 39.99 mg.kg<sup>-1</sup>, while the legislation allows a maximum HMF content of 40 mg.kg<sup>-1</sup>. In Spanish orange blossom honey, the HMF content was found to be from 25.49 to 59.84 mg.kg<sup>-1</sup> during storage. If this honey does not come from a tropical area, it exceeds the permitted limit of 40 mg.kg<sup>-1</sup>, but if it comes from tropical areas, the honey meets the requirements of the legislation (max. 80 mg.kg<sup>-1</sup>). In general, it can be stated that during storage at 4°C, there was a slower increase in HMF content than during storage at 20°C. During storage (3 and 6 months), honey darkened. By comparing the colour intensity during storage at different temperatures, it can be stated that when honey was stored at 20°C, it darkened faster than when stored at 4°C. During storage, there was a gradual decrease in free acidity and water content. Based on the results of our experiment, it is important to emphasize that when including all honey in the trade network, it is desirable that producers and distributors indicate on the packaging, in addition to the country of origin, its exact region of origin.

**Keywords:** honey quality, storage condition, HMF, electrical conductivity, free acidity

### INTRODUCTION

Findings of bee fossils from the Tertiary period found in Baltic amber indicate that bees inhabited Europe long ago. The use of bee products by humans is evidenced by the oldest evidence discovered so far in the form of cave drawings about the taking of honey from bee colonies. This period dates to the period before the emergence of agriculture. The age of these cave drawings is estimated to be at least eight thousand years. The first evidence of beekeeping comes from ancient Egypt, where they were kept in beehives made of clay, already four thousand years before our era (Chlebo, 2017).

Honey can be characterized as a supersaturated solution of carbohydrates with properties that are characteristic for each type of honey. Carbohydrates represent approximately 80% of honey, on average honey contains 38% fructose, 31% glucose and 11% complex carbohydrates. The remaining 20% is made up of water, which significantly affects the quality of honey, especially during its storage (Chlebo, 2017; Kamler, 2018).

Vyhľáška 41/2012 defines honey as a natural sweet substance produced by the honeybee (*Apis mellifera*) from the nectar of plants, excreta of living parts of plants or excrement of insects sucking living parts of plants, which bees collect, transform and enrich with their own specific substances, store, thicken, store and they leave it in the combs to ripen.

Blossom honey is obtained from the nectar of plants. Above all, it is nectar from meadow or other flowers and various fruit trees (Chen, 2019). Nectar is a sweet, sticky and sugary liquid, which is called insectivorous plants secrete by means of glandular formations designed to attract insects (Weiss, 2005). Honeydew is made by aphids that pierce the soft parts of the trees, digest the nitrogenous substances from the sap and excrete sucrose, which they cannot digest. Bees collect the secretions remaining on the plant and create honeydew honey (Korošec et al., 2016; Gonnet & Vache, 2018; Karabagias et al., 2020). Bees bring nectar or honey to the hive in honeycombs. In the hive, the bees sell the contents of the honeycomb to each other and enrich it with various enzymes contained in their saliva. Nectar or honeydew is stored in honeycomb cells where it ripens and at the same time evaporates water (Haragsim, 2016).

Honey contains more than 180 different substances, including carbohydrates, proteins, minerals, vitamins, and the like. The chemical composition of honey can be different depending on the environment, geographical conditions, and especially on the diversity of plants. Sensory and physico-chemical properties are mainly influenced by carbohydrates, which are the dominant component in honey (Nguyen, 2019).

Thanks to its properties, honey has been used for centuries: it is one of the oldest foods due to its sweet taste and health-promoting properties (Mahattanatawee et al., 2014). Honey is also used as a medicine for the treatment of various diseases, such as colds, wounds, coughs, diarrhoea, and other diseases (Taleuzzaman et al., 2020).

The physicochemical composition of honey is significantly affected by storage conditions. At low temperatures, based on studies, there are no significant changes in water content, on the contrary, at higher storage temperatures there is an increase due to the presence of a high content of metal catalysts and the enzyme glucoseoxidase, when glucose units decompose more quickly into gluconic acid and hydrogen peroxide. At the same time, hydrogen peroxide decomposes into water and oxygen, which increases the water content in honey. Storage at high temperatures reduces the water content due to honey maturation. Changes in water content (increase/decrease) in honey can also be caused by improper storage of honey (Manickavasagam et al. 2024).

In general, the higher the content of the above components, the higher the electrical conductivity of honey, and vice versa. Raweh et al. (2022) reported that it increased slightly (non-significantly) during eight months of storage at all storage temperatures (0–45°C). At higher storage temperatures (20°C), Zarei et al. (2019) found a non-significant increase in electrical conductivity, while Khan et al. (2021) found a statistically significant increase. However, Da Silva et al. (2020) found a significant decrease.

Usually, when honey is stored at both low and high temperatures, an increase in acidity occurs in most studies. In cases where a decrease in free acidity occurred, the authors assumed that it was due to the degradation of organic acids present in the honey (Manickavasagam et al. 2024).

Previous studies have shown that the increase in 5-HMF formation was greater at higher storage temperatures than at low storage temperatures. The authors assume that the insignificant difference in 5-HMF content at low storage temperatures (–20 °C and 4 °C) is due to the low reactivity of the reaction pathways of 5-HMF formation (Biluca et al., 2014; Manickavasagam et al. 2024).

The aim of this study was to determine the quality of foreign blossom honeys obtained from the trade network in Slovakia during storage.

**MATERIAL AND METHODS**

**Object of research**

For the analysis, 9 types of foreign blossom honeys were used, 5 pcs of each type. The honeys were purchased in a commercial network. Subsequently, we monitored the development of physico-chemical parameters at the beginning of storage, after the 3rd and 6th months of storage. The samples were stored in the dark at a room temperature (20°C) and at a temperature of 4°C.

Honeys (n = 45) of the following species and origin were used for the analysis of physico-chemical quality (Table 1). Based on the origin of the honeys, we considered honeys from citrus flowers (Italy - Sicily), from coffee tree flowers (Brazil), from tropical flowers (Cuba) and avocado flowers (Mexico) to be tropical. Other honeys were of non-tropical origin (Czech Republic, Germany, Italy, Hungary). Orange blossom honey from Spain could not be determined exactly, as it did not have a specific region of Spain listed on the packaging.

**Table 1** Honeys used in the experiment (n = 45)

Type of honey	Origin of honey
creamed blossom honey	Czech Republic
honey from citrus flowers	Italy - Sicily
honey from the flowers of the coffee tree	Brazil
honey from orange blossoms	Spain
blossom honey	Germany
chestnut honey	Italy
honey from tropical flowers	Cuba
false acacia honey ( <i>Robinia pseudoacacia</i> L.)	Hungary
avocado honey	Mexico

**Chemical analysis**

The following indicators were monitored within the physico-chemical analysis of honey:

- pH (desk pH meter Orion Star A211, Thermo Fischer Scientific, Waltham, USA) by puncture probe.
- electrical conductivity (it is expressed as the conductivity of a 20% solution of honey in water at a temperature of 20 °C, while 20% refers to the mixture without water. We calculated the weight of honey based on the dry matter content, determined using the refractometer Mini Digi Conductivity Meter OK-113 (Radelkis, Budapest). After preparing the sample, we determined the electrical conductivity on a conductometer (Conductivity meter Ok-113, Budapest). We calculated the electrical conductivity of the mixture solution as follows:

$$SH (mS.cm^{-1}) = K \times G$$

SH (mS.cm<sup>-1</sup>) – electrical conductivity of the solution mixture  
 K - electric cell constant  
 G (mS) – conductivity

- Colour intensity - spectrophotometrically (Spectrophotometer Jenway 7305, Cole-Parmer, United Kingdom), the method is based on measuring the absorption of radiation by different wavelengths - 450nm and 720nm. We diluted the honey in a 1:1 (w:w) ratio. We stirred the samples until the honey dissolved, filtered them, and then measured the absorbance on a spectrophotometer.

$$[AU] = (ABS450 - ABS720)$$

- Water content - we performed it using an Abbe refractometer (ATAGO Japan) according to IHC (2009). At a honey temperature higher or lower than 20 °C, we were adjusted the refractometric index using a correction. At temperatures above 20 °C we were added a correction of 0.00023 for each °C and at temperatures below 20 °C we subtracted a correction of 0.00023 for each °C.
- Total content of soluble solids – we performed it using a Re-Po-4 device (ATAGO, Japan) at a temperature of 20 °C.
- Free acidity - we carried out by the titration method, in which the consumption of 0.1 mol was monitored. dm<sup>-3</sup> NaOH to neutralize the free acids (FA) in the samples to the phenolphthalein indicator. To determine acidity, we took 10 g of honey sample, which we dissolved in 75 cm<sup>3</sup> of distilled water in an Erlenmeyer flask. After adding 2-3 drops of phenolphthalein, we titrated with sodium hydroxide solution producing a stable pale pink coloration with holding time 30 seconds.

$$FA [meq.kg^{-1}] = C [cm^3] \times 10$$

FA – free acidity [meq.kg<sup>-1</sup>]  
 C – consumption of 0.1 mol.dm<sup>-3</sup> sodium hydroxide

- Hydroxymethylfurfural (HMF) content – we carried out spectrophotometrically (Spectrophotometer Jenway 7305, Cole-Parmer, United Kingdom). Spectrophotometric determination of HMF is based on different absorbance of radiation. To prepare the sample, we dissolved 5 g of honey in distilled water in a 50 ml volumetric flask. Then we added 0.5 ml of Carrez I. solution, mixed, added 0.5 ml of Carrez II. solution, mixed again and made up to the mark with water. The solution was then filtered through filter paper and the first 10 ml of the filtrate was poured out. We prepared 4 test tubes for each sample. We took 5 ml of the filtrate into each test tube, 5 ml of distilled water (sample) was added to 3 test tubes and 0.20% sodium bisulfite solution (control) was added to the remaining 5 ml. We measured the absorbance compared to the control in 1 cm wide cuvettes at 284 nm and 336 nm.

$$HMF (mg.kg^{-1} \text{ honey}) = (A284 - A336) \times 74.87 / m \times 10$$

m = weight of the honey sample  
 A284, A336 = absorbance values at the respective wavelengths  
 Factor = 126 x 100 x 1000 x 100/16830 x 1000 = 74.87  
 126 = HMF molar mass  
 16,830 = molar absorptivity of HMF at a wavelength of 284 nm

**Statistical analysis**

The data obtained were subjected to analysis of variance (ANOVA) using SAS software (version 9.3, Enterprise Guide 4.2, USA). Tables show the results as the mean with standard deviation (SD) (SAS, 2008).

**RESULTS AND DISCUSSION**

The results of the physico-chemical properties of foreign blossom honeys are shown in Table 2 and Table 3.

**Table 2** Physico-chemical properties of foreign blossom honeys during storage at 20 °C (x±SD) and 4 °C (x±SD)

Type of honey/storage time	Chemical composition of honey during storage at 20 °C (x±SD)											
	Water content (%)			Total content of soluble solids (°Bx)			Free acidity (meq.kg <sup>-1</sup> )			HMF (mg.kg <sup>-1</sup> )		
	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month
Czech creamed blossom honey	19.86±0.05	18.26±0.05	17.16±0.05	78.32±0.16	80.08±0.08	80.96±0.11	15.01±0.22	11.98±0.61	12.00±0.35	2.241±0.255	5.178±0.553	15.270±0.284
honey from citrus flowers, Sicily	17.82±0.08	17.12±0.04	16.88±0.04	80.42±0.13	81.18±0.11	81.44±0.09	27.19±0.22	24.45±0.35	22.70±0.67	10.062±0.068	21.678±0.287	28.974±1.050
honey from the flowers of the coffee tree, Brazil	17.04±0.05	15.18±0.04	14.9±0.00	82.14±0.19	83.04±0.09	83.34±0.13	22.48±0.96	22.46±0.35	21.80±0.45	1.041±0.096	18.153±0.263	22.970±0.583
honey from orange blossoms, Spain	16.4±0.07	15.96±0.09	13.38±0.08	81.90±0.19	82.28±0.11	84.76±0.05	17.14±0.27	16.97±0.35	17.00±0.94	25.490±0.173	48.947±1.032	59.838±1.191
blossom honey, Germany	17.72±0.08	16.02±0.08	16.80±0.07	80.58±0.13	81.46±0.11	82.20±0.20	11.95±0.61	10.98±0.61	10.80±0.76	2.471±0.012	8.654±0.057	11.056±0.965
chestnut honey, Italy	15.96±0.15	15.6±0.10	13.56±0.09	82.28±0.24	82.56±0.05	84.60±0.10	26.95±0.42	23.45±0.50	23.50±0.61	1.895±0.083	5.662±0.014	11.549±0.531
honey from tropical flowers, Cuba	17.74±0.05	17.56±0.05	17.92±0.13	82.28±0.15	80.53±0.33	82.66±0.05	22.00±1.63	19.96±0.61	18.20±0.27	14.666±0.110	37.395±1.256	40.158±0.993
false acacia honey, Hungary	18.28±0.04	17.82±0.04	14.22±0.11	80.00±0.20	80.46±0.19	84.00±0.07	8.33±0.54	9.48±0.50	8.80±0.57	14.730±0.162	34.183±1.007	39.990±1.077

avocado honey, Mexico	16.48±0.13	14.9±0.07	13.74±0.09	81.14±0.09	83.42±0.04	84.40±0.10	27.98±0.65	26.45±0.35	26.40±0.42	0.594±0.084	6.822±0.355	10.681±0.821
<b>Chemical composition of honey during storage at 4 °C (x±SD)</b>												
Czech creamed blossom honey	19.86±0.05	17.4±0.13	17.3±0.13	78.32±0.16	78.90±0.10	80.98±0.15	15.01±0.22	12.00±0.5	11.97±0.61	2.241±0.255	1.796±0.784	9.055±0.482
honey from citrus flowers, Sicily	17.82±0.08	16.1±0.13	13.5±0.13	80.42±0.13	82.12±0.13	84.70±0.10	27.19±0.22	24.50±0.35	24.03±0.42	10.062±0.068	11.184±0.045	14.116±0.067
honey from the flowers of the coffee tree, Brazil	17.04±0.05	15.9±0.08	14.4±0.08	82.14±0.19	82.54±0.05	83.82±0.04	22.48±0.96	21.00±0.61	21.44±0.50	1.041±0.096	6.855±0.993	15.473±0.172
honey from orange blossoms, Spain	16.4±0.07	16.3±0.13	15.1±0.13	81.90±0.19	81.76±0.05	83.14±0.09	17.14±0.27	18.40±0.42	16.95±0.35	25.490±0.173	29.260±1.143	44.714±1.412
blossom honey, Germany	17.72±0.08	16.9±0.07	16.7±0.07	80.58±0.13	81.58±0.11	81.42±0.08	11.95±0.61	11.60±0.42	11.47±0.00	2.471±0.012	11.032±0.153	13.119±0.102
chestnut honey, Italy	15.96±0.15	15.9±0.08	15.8±0.08	82.28±0.24	81.50±0.10	82.32±0.08	26.95±0.42	24.90±0.22	24.53±0.22	1.895±0.083	3.218±0.460	25.206±0.243
honey from tropical flowers, Cuba	17.74±0.05	17.5±0.12	16.8±0.12	82.28±0.15	81.52±0.13	82.78±0.11	22.00±1.63	20.40±0.96	18.95±0.35	14.666±0.110	29.273±1.042	35.022±0.178
false acacia honey, Hungary	18.28±0.04	17.6±0.17	14.9±0.17	80.00±0.20	80.78±0.13	83.30±0.10	8.33±0.54	9.50±0.35	8.48±0.35	14.730±0.162	14.452±0.621	23.171±0.103
avocado honey, Mexico	16.48±0.13	15.9±0.12	12.8±0.12	81.14±0.09	80.70±0.10	85.40±0.10	27.98±0.65	25.49±0.71	30.01±0.89	0.594±0.084	9.107±0.312	14.308±0.602

<b>Physical properties of honey during storage at 20 °C (x±SD)</b>									
Type of honey/storage time	pH			Electrical conductivity (mS.cm <sup>-1</sup> )			Colour intensity (AU)		
	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month	0 day	3 <sup>rd</sup> month	6 <sup>th</sup> month
Czech creamed blossom honey	4.32±0.02	4.16±0.10	4.16±0.03	0.21±0.05	0.21±0.03	0.22±0.05	0.425±0.086	0.432±0.032	0.465±0.021
honey from citrus flowers, Sicily	3.48±0.01	3.61±0.17	3.79±0.09	0.35±0.02	0.35±0.03	0.43±0.02	0.473±0.054	0.459±0.071	0.458±0.032
honey from the flowers of the coffee tree, Brazil	4.22±0.02	4.32±0.09	4.42±0.09	0.45±0.03	0.45±0.02	0.50±0.03	0.692±0.111	0.71±0.049	0.724±0.052
honey from orange blossoms, Spain	3.98±0.01	3.98±0.06	4.16±0.09	0.25±0.02	0.26±0.04	0.28±0.03	0.521±0.055	0.536±0.087	0.566±0.040
blossom honey, Germany	4.82±0.12	4.84±0.17	4.76±0.07	0.49±0.04	0.60±0.04	0.50±0.07	0.595±0.123	0.609±0.036	0.589±0.074
chestnut honey, Italy	5.08±0.14	5.10±0.16	5.36±0.06	1.62±0.04	1.55±0.05	1.65±0.07	1.504±0.147	1.568±0.128	1.651±0.059
honey from tropical flowers, Cuba	3.93±0.05	3.90±0.03	3.84±0.08	0.32±0.02	0.36±0.01	0.38±0.06	0.601±0.085	0.612±0.035	0.601±0.060
false acacia honey, Hungary	4.06±0.09	4.01±0.11	4.15±0.11	0.14±0.03	0.15±0.03	0.15±0.05	0.196±0.032	0.218±0.012	0.225±0.012
avocado honey, Mexico	5.55±0.11	5.51±0.04	5.29±0.03	1.61±0.03	1.37±0.02	1.67±0.08	0.46±0.024	0.753±0.13	1.165±0.164

<b>Physical properties of honey during storage at 4 °C (x±SD)</b>									
Czech creamed blossom honey	4.32±0.02	4.10±0.07	3.89±0.06	0.21±0.05	0.19±0.03	0.21±0.03	0.425±0.086	0.423±0.065	0.412±0.022
honey from citrus flowers, Sicily	3.48±0.01	3.72±0.03	3.69±0.02	0.35±0.02	0.33±0.07	0.37±0.04	0.473±0.054	0.457±0.052	0.449±0.019
honey from the flowers of the coffee tree, Brazil	4.22±0.02	4.29±0.17	4.25±0.04	0.45±0.03	0.41±0.04	0.47±0.04	0.692±0.111	0.655±0.034	0.637±0.052
honey from orange blossoms, Spain	3.98±0.01	3.98±0.18	4.07±0.02	0.25±0.02	0.24±0.07	0.27±0.05	0.521±0.055	0.446±0.027	0.519±0.033
blossom honey, Germany	4.82±0.12	4.88±0.03	4.83±0.03	0.49±0.04	0.45±0.04	0.49±0.05	0.595±0.123	0.497±0.031	0.568±0.027
chestnut honey, Italy	5.08±0.14	5.20±0.07	5.26±0.02	1.62±0.04	1.49±0.03	1.66±0.16	1.504±0.147	1.738±0.109	1.54±0.169
honey from tropical flowers, Cuba	3.93±0.05	3.85±0.05	3.95±0.04	0.32±0.02	0.33±0.06	0.36±0.04	0.601±0.085	0.599±0.067	0.595±0.079
false acacia honey, Hungary	4.06±0.09	3.91±0.03	4.20±0.06	0.14±0.03	0.17±0.03	0.15±0.05	0.196±0.032	0.198±0.012	0.195±0.024
avocado honey, Mexico	5.55±0.11	5.87±0.04	5.61±0.01	1.61±0.03	1.46±0.03	1.65±0.12	0.46±0.024	0.728±0.046	1.321±0.145

**Water content**

The water content affects the shelf life of honey (Scripcă, 2019) and depends on the beekeeper's ability to estimate the time of honey extraction (Goro, 2020). The water content of honey is influenced by many factors, for example the place of storage, or time and temperature of honey storage (Balážová, 2014). Our results also confirm the statement of Vijayakumar (2021) that the water content by storage at a temperature of 20 or 4 °C decreased, while at the beginning of the experiment (day 0) Czech creamed honey reached the highest value (19.86%) and chestnut honey from Italy (15.96%) the lowest. After storage (6<sup>th</sup> month) at a temperature of 20 °C, the highest value was again achieved by Czech creamed honey (17.30%) and the lowest by orange blossom honey from Spain (13.38%). By storing honey (6<sup>th</sup> month) at a temperature of 4 °C, the water content was the highest in Czech creamed honey (17.30%) and the lowest in avocado honey from Mexico (12.80%). All monitored honey samples met the legislative requirements for honey (Council Directive 2001/110/EC; Vyhláška 41/2012) Water content is an important factor in determining the shelf life and maturity level of honey. Too much water in honey leads to fermentation, spoilage and loss of taste, all of which reduce the quality of the honey. Certain types of honey can crystallize more quickly when the moisture concentration is high, and their water activity can rise to the point where some yeasts can function and the fermentation that occurs is brought to Ouchemoukh et al. (2007), and Saxena (2010).

**Total soluble solids content**

Honey contains approximately 75% monosaccharides and 10 – 15% disaccharides with other carbohydrates. The high sugar content in honey causes its texture to be more viscous and therefore honey is more hygroscopic. Honey with a high sugar content affects the colour, taste, and shelf life of honey (Evahelda et al., 2017). The lowest total content of soluble solids in our experiment was at the beginning of storage (day 0) 78.32 °Bx (Czech creamed honey) at a temperature of 20 °C. At the end of storage (6<sup>th</sup> month) at a temperature of 20 °C, the total content of soluble substances the most increased to 84.76 °Bx in honey from orange blossoms from Spain. The highest value of soluble solids was after 6 months of honey storage at a temperature of 4 °C (85.40 °Bx) in avocado honey from Mexico. The increase in the total content of soluble solids during storage at both observed temperatures (4 and 20 °C, respectively) is in accordance with the findings of Minhas and Dhaliwal (2018).

**Free acidity**

The free acidity of honey is mainly caused by organic acids, even though their content is lower than 0.5%. Gluconic acid, which is the result of the action of honey glucose oxidase on glucose, contributes mainly to the acidity of honey. Gluconic acid is in equilibrium with gluconolactone. The acidity of honey is also caused by other organic acids together with inorganic anions. The content of total free acidity can vary considerably. Free acidity contributes to honey flavour, stability against

microorganisms, promotes chemical reactions, and has antibacterial and antioxidant effects (Cavia et al., 2007). The content of free acidity in honey is highly variable and depends on the origin of the honey. The highest acid content on day 0 was in avocado honey from Mexico (27.98 meq.kg<sup>-1</sup>) and after storage (6 months) at a temperature of 20 or 4 °C was also for this observed honey (26.40, respectively 30.01 meq.kg<sup>-1</sup>), which is by the results of Rodríguez et al. (2019). The lowest values of free acidity of honey were achieved during storage by blossom honey from Germany (10.80 meq.kg<sup>-1</sup>), which corresponds to the results of Adgaba et al. (2020) or Živkov et al. (2020). Higher values of free acidity of honey (20.32 - 27.19 meq.kg<sup>-1</sup>) were achieved by honey from tropical flowers (Cuba), honey from coffee tree flowers (Brazil), honey from citrus flowers (Sicily), which is also confirmed in their results by Jalili et al. (2023). In the end, all monitored honey met the limited values of free acids (50 meq.kg<sup>-1</sup>) according to Council Directive 2001/110/EC, and Vyhláška 41/2012.

## HMF

The HMF content is a precursor of honey quality. The formation of the cyclic aldehyde 5-hydroxymethylfurfural (HMF) occurs in an acidic environment through the Maillard reaction from reducing carbohydrates, possibly also during caramelization. A non-enzymatic browning reaction that leads to an increased level of HMF can occur during food processing, such as heating or long or improper storage. HMF has been attributed to many adverse effects on human and animal health, such as cytotoxicity, mutagenicity, carcinogenicity or chromosomal aberrations. Increased concentrations of HMF in honey or sugar fed to bees can cause dysentery and ulceration of the intestines of bees as well as themselves (Sajtos et al., 2024). On day 0, the highest content of HMF (25.49 mg.kg<sup>-1</sup>) was in honey from orange blossoms (Spain) and the lowest in avocado honey from Mexico (0.59 mg.kg<sup>-1</sup>), which agrees with the findings of Rodríguez et al. (2019). During storage for 6 months at a temperature of 20 or 4 °C, lower HMF values were mostly achieved by storing honeys at 4 °C. During storage at 20 °C, honeys reached an increase in HMF from 2.24 to 15.27 mg.kg<sup>-1</sup> in Czech creamed blossom honey, which can be attributed to the method of treatment of honey, namely its creaming. The most significant increase in HMF during 6 months of storage at 20 °C was recorded with honey from tropical flowers, but it did not exceed 80 mg.kg<sup>-1</sup> (Council Directive 2001/110/EC, and Vyhláška 41/2012). In orange blossom honey from Spain, the HMF content during storage was from 25.49 to 59.84 mg.kg<sup>-1</sup>, from tropical areas, meets the requirements of the legislation (max. 80 mg.kg<sup>-1</sup>). From this point of view, it is important that all producers and distributors indicate the exact region of origin of honey on the packaging, so that the honey can be assessed with certainty according to the legislation, as this is not it is possible to determine whether it is honey from a tropical area.

Agave honey after storage at 20 °C reached the threshold value of 39.99 mg.kg<sup>-1</sup>. Based on the obtained results, we can agree with Sajtos et al. (2024), who recommend the consumption period of honey from the point of view of HMF content between 6 months and up to 1 year. Monitored agave and orange blossom honeys pose a potential safety risk from long-term storage due to HMF content. Sajtos et al. (2024) state that after 5–6 years of proper storage, e.g. agave honeys will reach the consumption limit of HMF concentration, however, they recommend the most suitable period of consumption of honey from the point of view of HMF content within 6 months and up to 1 year.

## pH

Council Directive 2001/110/EC and Vyhláška 41/2012 not determining the pH value of honey. However, the pH value of honey is associated with the presence and growth of microorganisms, where a low pH prevents microbiological spoilage (Ratiu et al., 2020). The pH of honey also depends on ionized acids and the content of mineral elements. The pH of honey influences texture and enzymatic activity (Cavia et al., 2007). The pH value in our experiment was from 3.48 to 5.87. Similar results were also achieved by Ratiu et al. (2020) multi-floral Greece honey with a pH of 4.30 or 4.27, false acacia Romania honey pH 3.80, false acacia Poland honey pH 3.61 or 3.58; Adgaba et al. (2020) in multi-floral honey from Ethiopia 4.1; Albu et al. (2021) in false acacia Romania honey 3.675; Szeles et al. (2006) in false acacia Hungarian honey 2.82 – 3.41; Bodor et al. (2019) in false acacia honey 3.9; Rodríguez et al. (2019) in avocado Spanish honey 4.3 – 6.2. The low pH value is caused by a high concentration of organic acids (e.g. gluconic acid). The pH of most types of honey is in the range from 3.4 to 6.1, which, in combination with high osmotic pressure, eliminates or makes impossible the development of most microorganisms (Szweda, 2017).

## Electrical conductivity

The electrical conductivity of honey is considered a very good criterion for evaluating the botanical origin and purity of honey. In general, it can be said that light honey usually has a lower conductivity than dark honey. Electrical conductivity is determined by the content of substances that, in an aqueous solution, can dissociate into ions or conduct electricity (Kropf et al., 2008; Živkov et al., 2018). Honeydew and dark honeys are characterized by a higher content of

mineral substances, which corresponds to a higher electrical conductivity value of such honeys. Blossom honeys are characterized by a lower content of mineral substances and thus have a lower final value of electrical conductivity (Čuboň et al., 2012). The electrical conductivity of honey has a strong correlation with the concentration of mineral salts such as potassium, calcium, copper, iron, magnesium. Electrical conductivity also depends on the content of organic acids and proteins. The high variability of electrical conductivity enables identification of the botanical origin of honeys and their identification into blossom and honeydew honeys (Majewska et al., 2019). Tropical avocado honey from Mexico (1.61 mS.cm<sup>-1</sup>) achieved the highest values at different honey storage times and temperatures (1.67 mS.cm<sup>-1</sup>), which is higher than the requirement of Council Directive 2001/110/EC and Vyhláška No. 41/2012, which sets a maximum limit of 0.8 mS.cm<sup>-1</sup>. The results of electrical conductivity, which were from 1.46 to 1.67 mS.cm<sup>-1</sup>, were also found by Orantes Bermejo & Gómez Pajuelo (2007). Terrab & Heredia (2004), and Rodríguez et al. (2019) who reported values in Spanish avocado honey of 0.6 – 1.4 mS.cm<sup>-1</sup>. In agate honey, we found electrical conductivity from 0.14 – 0.17 mS.cm<sup>-1</sup>, like Živkov et al. (2020) in agate honey from Serbia (0.18 mS.cm<sup>-1</sup>). Decree 41/2012 sets a maximum electrical conductivity limit of 0.8 mS.cm<sup>-1</sup> for blossom honeys, except for chestnut honey, where the limit value is at least 0.8 mS.cm<sup>-1</sup>. When comparing the values of the electrical conductivity of the analyzed samples with the legislation of the SR, we can conclude that all samples, except for avocado honey, met the requirements of the legislation.

## Colour intensity

Kek et al. (2014) state that the colour intensity of honey has a positive correlation with the antioxidant potential of honey. The values of honey colour intensity during storage (425 – 1738 mAU) found by us were similar to Romanian and Indian honeys, which were in the range of 210 to 1228 mAU and 524 to 1678 mAU (Saxena et al., 2010; Cimpoi et al., 2013). In general, it can be concluded that storage of honeys at temperatures of 20 °C caused them to darken more significantly than when stored at room temperatures of 4 °C.

## CONCLUSION

The evaluation of the quality of foreign honeys from the Slovak trade network proved that not all honeys meet the requirements of Decree 41/2012 as well as Council Directive 2001/110/EC. The examined avocado honeys did not meet the requirements of the maximum limit of electrical conductivity of 0.8 mS.cm<sup>-1</sup> and the agave honeys reached the limit value of HMF content (39.99 mg.kg<sup>-1</sup>) after 6 months of storage at room temperature. Such honeys cannot be recommended for longer storage. It was not possible to evaluate the HMF content in the case of honeys from orange blossoms originating in Spain, as it was not possible to classify this honey as tropical or non-tropical honeys. Based on these findings, we would recommend that in honeys that come from foreign regions, it should always be stated on the label whether it is tropical honey or not, as orange trees in Spain are grown in the Mediterranean as well as in tropical regions. In conclusion, it can be concluded that physical and chemical properties are the cornerstone on the way to assessing the quality of honey and identifying its authenticity and origin.

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